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ON THE VARIATIONS OF LEUCOCYTOSIS

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ON THE VARIATIONS OF LEUCOCYTOSIS

by

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(See Table II)

ABSTRACT. Artificial changes of leucocyte count in the blood are examined in extensive experiments with rabbits. Some of the older (19th century) theories regarding leucotasis and leucocytes are reviewed. Numerous substances were injected into various organs and the changes in the count were observed and analyzed. Some of the older theories are refuted on the basis of the described experiments. The application to human subjects is discussed.

Numerous articles on blood, either from a clinical or experimental physiological viewpoint, have appeared in the last years. A rather exhaustive survey on all these works as far as they relate to leucocytosis appeared 1891 in a book by Reinert [1] and in the following year by Rieder [2]. Therefore, it is not necessary to go into all of this literature.

In recent years primarily the question of artificial changes of leucocyte count was our concern. We tried to discover its nature and test present views on this. The results of our experimental investigations were partially communicated in two lectures given last year in the Physiological Society in Berlin, but we were confined to a brief survey which allowed only a sketch of our experiments. However, we deem it necessary to present the information in its entirety.

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*Numbers in the margin indicate pagination in the original foreign text.

Disregarding views to be developed later, the following views on the nature of leucocytosis are prevalent: by v Limbeck, Buchner, Römer, Löwit and Schulz. Limbeck's theory [5] connects leucocytosis intimately with the formation of exudate and considers the latter only an accompanying phenomenon of the former. He bases these views on the following considerations of experiments. Microbial metabolic products introduced into the animal body in varying doses produce reactions depending on the quantity. Neither the blood /374 nor the remaining tissues of the animal react to small quantities; larger quantities, however, produce local reactions by exudation and moderate hyperleucocytosis. A considerably more intensive reaction of the tissues and a much stronger hyperleucocytosis is produced when viable pus micrococii settle in the body. Limbeck thinks hyperleucocytosis is caused by metabolic products of the microorganisms, or rather the microorganisms themselves exert a remote effect upon the white portion of the blood and force them in large quantities into the circulatory system. Limbeck cannot give an explanation for this remote effect and cannot decide whether hyperleucocytosis increases the production of cells in the blood producing organs like spleen, bone marrow, lymph glands, or only removes them from such organs; however, he indicates chemotactic influences which probably play a part in these processes.

Römer [6 and 7] proposes a second theory in connection with his experiments of bacterial proteins in rabbits which he and Buchner [8 and 9], in part he alone, performed. According to Römer, hyperleucocytosis is produced by decomposition products of dead bacteria or other cells, particularly proteins, reaching the tissue fluid, then the lymphatic system and the blood where they exert a direct formative stimulus on the white blood corpuscles. According to Römer, multiplication is effected by amitosis. He, therefore, contests that the increased cells found in cases of hyperleucocytosis are already present in blood producing organs and have reached the blood stream as a consequence of chemotactic influences, but thinks that these influences act directly on the leucocytes.

Löwit's theory [10] about leucocytosis is entirely divergent from this. He conceives of the process of hyperleucocytosis as entirely independent from

chemotaxis and believes that it is merely a previous reduction of leucocytes in the blood. Accordingly, he supposes hyperleucocytosis arises whenever leucocytes have partially disappeared from the blood, thereby causing an increased influx of young leucocytes from the organs producing blood cells. /375
Löwit arrived at this conclusion after a great many experiments with rabbits. He found that hyperleucocytosis occurred in connection with leucopenia and leucolysis; the former term designates the reduction of leucocytes when the experimental animal is cooled, the latter the presumed dissolution, supposedly the consequence of certain injections. The injections contained bacterial proteins, hemialbumose, peptone, pepsin, nucleic acid, nuclein, blood gel extract, curare, uric acid and urate sodium bicarbonate. In all cases he found a significant reduction of leucocytes following the injection, but then a gradual increase in proportion to the previous decrease. According to Löwit, hypo- and hyperleucocytosis are two inseparable processes, the second emerging from the first.

A fourth theory on leucocytosis is proposed by Schulz [11]. He believes that white blood corpuscles do not decrease or increase under any conditions, considered to be hypo- and hyperleucocytic, but are merely distributed differently in the vascular system. According to Schulz the injection of protein and other materials causes neither a deterioration of leucocytes, the leucolysis of Löwit, nor a subsequent absolute increase of leucocytes from the blood producing organs.

We are satisfied with having briefly indicated the views which we encountered at the start and during our investigations, since we have to examine the details later upon which these theories are founded; for in order to formulate our own views on the nature of leucocytes we had to examine the correctness of the existing ones by analogous investigations. We have concerned ourselves particularly with the teachings of Schulz and Löwit and from these investigations gained a clue to the direction in which the experiments should be continued in order to formulate our own views on the nature of leucocytosis and to provide more evidence for them.

Experimental Procedure

In order to be more brief in our report we will first give a general account of our procedure. Rabbits were used exclusively. They have the /376 advantage, as shown in an earlier test, of not being subject to periodic variations in white blood cell count (Pohl, Schulz, Jacob). The leucocyte count, however, varies greatly with the individual animal (between 8,000 - 14,000); therefore, we always count the number of white blood cells before each experiment in order to be able to judge the magnitude of the change caused by the injections. For nearly every experiment we used a new, unused animal; we waited at least 3 weeks before we used a previously employed animal for a new experiment. We considered this inevitably necessary to maintain the purity of the experiment, since the wound, even though all infection is avoided, and especially the loss of blood and the loss of appetite during the first couple of days after the operation has an important influence on leucocytosis. The blood for the counts was generally taken from the peripheral vein of the ear after the ear had been shaven and cleansed with ether. For experiments using intravenous injection the blood sample was taken from a blood vessel in the thigh (the femoral artery or vein) which was exposed and clamped off enabling us to collect blood most speedily for hours. In the meantime the wound was naturally well covered. The count was done on a Zeiss-Thomas counting apparatus and diluted 1:20 with 1/3 per cent acetic acid when we only wanted to count the leucocytes. When erythrocytes were to be counted also, we used the usual physiological saline solution or the Toison mixture. To determine the number of leucocytes, the 400 squares of the counting chamber were counted, for erythrocytes twice sixteen squares. Whenever possible we collected two successive blood samples from different capillaries and counted in the above manner. Errors, in our experience, amounted to 200-800 for a normal count of 8,000, i.e., 1-4 for each of the 400 squares of the counting chamber. Frequently, in the course of the experiments we prepared blood samples immediately after the count and treated them with triacid by the method described by Ehrlich.

The injections were given subcutaneously into the skin of the abdomen which had been shaven and disinfected. The intravenous injections were administered into one of the external jugular veins; in the rabbit these are well developed and are situated rather superficially so that it does not require an extensive incision to find it and to fasten a canula in it. When the animals were not sacrificed immediately after the experiment but saved for counting within the next few days or for other experiments, the wound was stitched after disinfecting it thoroughly with alcohol and was closed with iodoform collodium. In every other respect the experiments were executed under strictest aseptic precautions, i.e., before the experiment the site of operation was disinfected, similarly all instruments and utensils, as well as the wound itself, being covered with aseptic material for the duration of the operation. Under these conditions, it is usually possible to avoid any infection of the wound which is of prime significance, for if purulence should occur, every count taken during the particular experiment is completely irrelevant since it is impossible to decide whether the present hyperleucocytosis is the result of the particular injection, the combination with the suppuration, or the result of the latter alone. /377

Singular, subcutaneous injections, quickly executed, obviously did not require narcosis; however, experiments using intravenous injection and lasting several hours made narcosis inevitable. Initially, we used chloralhydrates, but encountered frequent difficulties. Since we could not attain a reliable, uniform dosage in all cases, we soon discontinued using chloralhydrate altogether and used the older method of ether narcosis exclusively. We would like to point out that the narcosis has to be observed carefully because the animals wake up with a sudden sharp jerk and might possibly upset the experiment considerably. As soon as the animals had been put to sleep in this manner, they were fastened on the rabbit board. After we had tested Löwit's results that the experimental animal being tied down for hours cools off and the number of leucocytes is considerably reduced and we had verified this, we wrapped the animal in warm cloths for the duration of the first experiments in order to avoid the trouble of cooling. But the procedure is very cumbersome. Therefore, we have modified a rabbit board on which the animals were /378

tied during the experiment so that a metal box was fastened under the box which rested on tall iron legs. About half an hour before the experiment, the box is filled with sand and heated with a flame underneath to ca. 40° (centigrade). The flame is then extinguished, the temperature of the sand increases by a few more degrees and remains rather constant when during the experiment frequently a very small flame is placed under it. The temperature of the experimental animal which is tied on the rabbit board remains equally constant. We will show later on in several experiments that by these measures leucopeny mentioned by Löwit is avoided entirely.

As already mentioned, a great deal of our work consisted of examining the organs of the experimental animals partly in a fresh condition, partly in a hardened condition. We mostly examined heart and lungs, sometimes also liver, spleen and bone marrow. The thoroughly anesthetized animal had its thorax and abdomen opened, ligatures applied about the major vessels and the organs removed. The fixing fluid was partly Müller's solution, partly absolute alcohol, mostly Müller's solution and absolute alcohol, or Müller's solution and sublimate solution, concentrated. When the organs had been hardened in the last solution, it was found to be advantageous to add several drops of tincture of iodine to the alcohol which prevents sublimate precipitation. The organs were either put in paraffin or "celloidin", and the preparations stained with Biondi's stain mixture or with Ehrlich's triacid.

For the injection experiments we used substances used in part already by other authors. We injected hemialbumose, obtained from Dr. Grübler in Leipzig, as Löwit had done, nucleic acid, courtesy Prof. Kossel, tuberculin, other bacterial proteins, prepared by Römer's process [12], etc. Moreover, did we inject our own carbolic acid glycerine extracts prepared from organic tissues. For the sake of completeness we would like to describe the method of preparation of these substances, as we did already in one of our above mentioned /379
lectures. Presently our observations were concerned with the spleen, thymus, bone marrow, pancreas, thyroid, liver, kidney, mostly from young calves. The organs are sent from the central stock yard to the laboratory quite fresh in the morning where they are immediately processed. The instruments and vessels

used for this naturally have to be completely sterile. The organ is thoroughly rinsed in water, removing all surrounding fat and connective tissue. Another cleaning in water is followed by a ca. 3% carbolic acid solution. The preparation is then placed on a sterile glass plate and its entire surface removed by a single cut with a long sharp knife, or to obtain the bone marrow, the bone is sawed apart lengthwise. Pieces are immediately cut with scissors and forceps from the center of the organ and thrown into a porcellan mortar. It contains already the necessary quantity of glycerin, purified, mixed with an aqueous solution of carbolic acid. The pieces are macerated by using small pieces of glass which cut up the substance to an extreme degree. The extract thus obtained is poured into a glass container and kept in the refrigerator for 24 hours. Then it is strained through a piece of linen, sterilized by repeated boiling, into another glass container, requiring special asepsis of the hands. Most extracts are still not clear enough and are strained through two or three additional linen pieces. The extracts are stored in glass containers with sterilized cotton plugs in a cool place and keep excellently for a long time. After weeks we convinced ourselves of their sterility by inoculating nutrient agar plates and broth. As a matter of fact, we usually spread some of the extract, before using it for injection, on nutrient media and begin our experiments only if nothing had grown after 2-3 days. Most recently, Dr. Krüger and the second author, working in Dr. Kossel's laboratory of the physiological Institute in Berlin, have commenced to analyze and test the chemical nature and physiological effect of the leukocytogenic substance contained in the carbolic acid-glycerine extract. However, the experiments are not yet concluded and will be mentioned later on.

At the conclusion of this section we would like once more to mention and suggest the nomenclature which we have used for some time to express the changes in the quantity of leukocytes, as postulated in our first lecture. /380
Löwit, as already mentioned above, calls the reduction of leukocytes in the blood due to various circumstances either leukopenia or leukolysis. We would like to suggest the collective term hypoleukocytosis, especially since so many investigators conceive of white blood corpuscle reduction quite differently from Löwit. Conversely, every increase is to be called hyperleukocytosis,

while the old term leukocytosis would be suitable to express the leukocyte quantity in general. In the French literature, as a matter of fact, this expression is sometimes equally common.

Dr. Robert Franz Müller has kindly helped us frequently in the following experiments, enabling us to make the large number of blood counts in such quick succession. We herewith express our thanks to him.

Influence of Cooling and Shock

In the following experiments we first wish to show what influence gradual cooling has on leukocytosis and how differently the results turn out when under the same experimental conditions the animal is held at rather constant temperature.

These experiments were conducted merely to determine the leukocyte number during cooling and tying down, and they show clearly that prolonged and increased cooling intensifies hypoleukocytosis. In this respect we can only verify Löwit's results, but on some points we disagree. For one thing, we were not able to convince ourselves that reduction to one half of the count was due to continuing fettering, as in one of Löwit's tests (34567-18403). We believe that this observation came about by taking the first count before tying the animal down, from blood in a small ear artery, the second, however, from the jugular vein; a very important circumstance, as we shall see later on. Löwit himself admits that fettering alone "produces only a sudden relatively low grade reduction of the number of leukocytes". As far as the reduction of the number of leukocytes due to cooling is concerned, we do agree with Löwit in that, by avoiding cooling, leukocytosis remains at about the same level. However, when the body temperature of the experimental animal drops increasingly, the number of leukocytes drops, at least in the vessels which are accessible to us for blood samples and count. However, we differ decidedly with Löwit in the interpretation of these phenomena. Löwit distinguishes completely between hypoleukocytosis caused by prolonged fettering,

Exp. No.	Manner of action	Time of day hr min	Temp. °Cent	Number of leukocytes* /cmm from fem. vein	General Comments
		Feb. 12			
1.	Rabbit slowly tied operated	11:30am	38.2	Ear vein 10600	Rabbit is anesthetized, lies uncovered, tied to regular board; one fem. vein exposed, also jug. vein. Wounds covered with sterile gauze.
		11:45am	38.0	7200	
		12:10pm	37.3	6000	
		12:40pm	34.8	4800	
		1:10pm	33.6	4000	
		1:35pm	32.4	3800	
		2:5 pm	31.8	3400 !	Blood slides at 2:5 pm show mostly polynuclear eosinophil and several mononuclear cells.
		2:25pm	31.6	3600	
		6:40pm	37.8	Ear vein 13800	
		Feb. 13			
		10:5 am	38.0	Ear vein 14600	The animal's incisions are closed up, it is wrapped in warm cloths and allowed to recuperate.
		Feb. 13			
2.	Rabbit operated and slowly tied	11:50am	37.9	Ear vein 9400	As in 1. /381
		12:5 pm	37.7	6200	The slides made from the blood of the ear vein before tying, show ca. 60% polynuclear and 40% mononuclear cells.
		12:10pm	37.4	7000	
		12:15pm	37.2	6400	
		12:45pm	36.1	5600	
		1:10pm	34.0	4600	
		1:45pm	32.8	4000	The blood slides made about 1:45pm show again predominantly polynuclear eosinophil cells.
		2:5 pm	32.9	3800	
		2:30pm	31.2	3000	The animal is sacrificed.
		Apr. 2			
3.		12:00am	38.9	Ear vein 9600	Rabbit is slowly cooled by water irrigation.
		12:37pm	29.5		
		12:54pm	25.2	Ear vein 3400	Animal is killed for microscopic investigations.

* Unless otherwise indicated, the number of leukocytes refers to the count made from the femoral vein.

Exp. No.	Manner of action	Time of day hr min	Temp. °Cent	Number of leukocytes /cmm from fem. vein	General Comments
		Oct. 17			
4.	Rabbit is tied and operated	12:5 pm	38.4	Ear vein 10600	Rabbit is anesthetized, lies on warmed board. One ext. jug. vein and one fem. vein are exposed.
		12:25pm	38.1	8600	
		1:00pm	38.0	8800	
		1:20pm	38.0	8400	
		1:45pm	38.1	8800	
		2:5 pm	38.0	8200	Blood slides made at about 2:5 pm show the usual polynuclear and mononuclear leukocytes in a normal relationship.
		2:25pm	37.9	8400	
		6:40pm	—	Ear vein 11000	
		Oct. 18			
		9:5 am	38.2	Ear vein 10800	
		Sept.15			
5.	Rabbit is tied and operated	11:30am	38.2	Ear vein 11200	Rabbit is anesthetized, lies on the board wrapped in warm cloths; an ext. jug. vein are exposed.
		11:55am	37.9	8200	
		12:10pm	38.0	8600	
		12:45pm	37.8	7800	
		1:10pm	37.6	7400	
		1:35pm	37.6	7800	
		2:15pm	37.5	7400	
		6:10pm	38.1	Ear vein 11800	
		9:30am	38.3	Ear vein 11000	
		Mar. 25			
6.	Rabbit is tied and operated	11:00am	38.0	Ear vein 10200	Rabbit lies on warmed board, unanesthetized, only tied; an ext. jug. vein and a fem. vein are exposed.
		11:30am	37.8	7000	
		11:50am	37.8	7400	
		12:15pm	37.7	7800	
		12:40pm	37.7	7400	
		1:5 pm	37.7	7000	
		1:40pm	37.6	7000	
		Apr. 2			/382
7.	Shock by striking neck	1:5 pm	—	Ear vein 10400	
		1:10pm	—	Shock	
		1:11pm	—	Ear vein 4200	Animal is killed for microscopic examination.
		Apr. 9			
8.	Shock by striking abdomen	12:5 pm	—	Ear vein 10800	After the shock the animal is unconscious for about 5 min. Heart rate rises from 240 to 358 beats/min.
		12:7 pm	—	Shock	
		12:8 pm	—	Ear vein 3800	

and that which results from cooling: the former is assumed to come about since, as a consequence of fettering, there is an actual decline of leukocytes, both mononuclear and polynuclear, and, furthermore, the supply of immature /383 mononuclear elements from the organs producing blood cells is reduced. But hypoleukocytosis caused by cooling, called leukopenia, he considers merely as an inadequate supply of leukocyte-like elements to the blood, and he believes that in this type of hypoleukocytosis destruction of leukocytes can only be involved to a certain degree. We do not agree with this explanation of hypoleukocytosis caused by cooling and fettering. We were not able to detect a considerable reduction in the number of leukocytes solely due to fettering when cooling was avoided by the method described above. Löwit indicates this fact himself: "When the animal was not allowed to cool by wrapping it in poor heat conductors, or at least considerably retarding the cooling process, the influence of prolonged restraining on the number of leukocytes was either completely absent or noticeable only to a very slight degree." However, we noticed that the leukocyte count in blood from the femoral vein was constantly less than in the blood from a little ear vein, a relationship which will be explained in one of the later chapters. But leukocytosis remained at the same level when the experimental animal was kept at constant temperature. Also, the ether anesthesia, as is shown in experiment No. 6, does not influence this process in the least since we arrived at the same results without it. Since anesthesia is no source of error, but restraining ropes, drawn too tightly or sudden movement of the experimental animal may well be, we have used anesthesia in almost all of our experiments. From this one can only interpret the results of Löwit's experiments, in which he reports that after two to three hours of restraining the experimental animal, its leukocyte count had dropped to one half, one third or less, to mean that this drop was the result of cooling during the hours of restraining. This we would like to stress in contrast to Löwit: Prolonged restraining alone does not cause hypoleukocytosis, only in connection with cooling or rather the latter by itself.

Now the question is, what causes this kind of hypoleukocytosis. Löwit explains the sudden reduction of leukocytes in the blood as a consequence of

restraining, as the effect of shock. In his opinion the same result is achieved by certain other actions, for instance, strong blows on the neck, of such force as to effect mild nystagmus and temporary paralysis of the extremi- /384
ties, and this causes a sudden destruction of leukocytes. On the other hand, Löwit does not consider hypoleukocytosis due to sudden cooling a shock effect. Should such be the case, then a rapid cooling must be considered at least equivalent with restraining which also takes some time. To investigate this question, we have to make a distinction, which Löwit ignores, between hypoleukocytosis as a consequence of prolonged cooling and the one as a consequence of shock effect, e.g., sudden cooling, restraining, blows on the neck.

We shall first deal with the question of what takes place in hypoleukocytosis brought about by hours of cooling; if, indeed, an actual destruction of the leukocytes occurs or only a reduced influx of immature leukocytic elements from the organs producing the blood cells, or if there is ultimately another reason. Löwit assumes the first two causes, i.e., destruction in hypoleukocytosis as a consequence of prolonged restraint, and reduced influx during cooling. We have shown above that cooling and restraint are inseparable; if Löwit's hypothesis is justified, both circumstances — cooling, as well as restraint — must produce both destruction and reduced influx. As evidence for his assumption that restraint produces a decline of leukocytes, Löwit states that hyperleukocytosis comes about and that it is possible to achieve intravascular thrombi. These two facts are verifiable by our test record, but at the same time we point out that the same things happen after cooling has been survived. Only later on can we establish whether this hyperleukocytosis actually is evidence for the destruction of white blood corpuscles in hypoleukocytosis due to cooling and restraint.

Concerning the second reason for hypoleukocytosis by cooling and restraint, which Löwit sees in the reduced influx of immature leukocytes from the organs forming the blood cells, he relies on the observation that during this hypoleukocytosis primarily the number of mononuclear leukocytes decreases. It is that number which he takes as the measure for the influx of /385
immature white blood corpuscles. This point we will have to discuss in more

detail in a later chapter. We would like to point out one fact contrary to Löwit's findings: after the experimental animal has been tied down and the slight variation in the leukocytosis, probably caused by the restraining, is removed, it is found that the polynuclear leukocytes definitely outnumber the mononuclear ones, by 65% to 35% of the total. Löwit indicates that this relationship changes during prolonged cooling insofar as the number of polynuclear leukocytes is not supposed to decrease by far to the degree by which the mononuclear ones reduce. According to our investigations this statement requires considerable qualification which makes Löwit's hypothesis very unstable. The same applies to the form of the polynuclear ones. In the normal animal we usually find the normal polynuclear cells before cooling; after hours of cooling these are almost completely absent; eosinophilic, mostly polynuclear leukocytes are in their place. For the moment, we only indicate this finding, and at the end of the paper we will draw conclusions and determine whether Löwit's hypothesis still remains valid — namely, hypoleukocytosis due to cooling is caused by decreased influx of immature leukocytic elements from organs forming blood cells.

Finally, we will discuss the shock effect, including strong blows on the neck, as well as restraint and sudden cooling. We also cannot agree with Löwit on the interpretation of hypoleukocytosis caused by shock because our findings contrast with his. Löwit, in an earlier dissertation has mentioned that in the normal rabbit the number of polynuclear cells is much larger than the number of mononuclear cells. He qualifies this in his last work by stating that the particular relationship between poly- and mononuclear white blood corpuscles only exists in restrained rabbits, while the opposite should be the case when unrestrained. We cannot concur with him in this statement. Even if polynuclear leukocytes do not predominate over mononuclear ones in the rabbit to the degree in which they do in man, nevertheless, we found the former always in greater number than the latter in our test animals also when we avoided any restraining, any shock effect. On the other hand, we found, contrary to Löwit, that hypoleukocytosis by shock produced mainly large /386 polynuclear and especially eosinophilic cells. As for the rest, we can agree with Löwit's results that it is possible to produce hypoleukocytosis by shock

without, for the time being, dealing with the question of whether in this case the vasomotor influences play a part, as Löwit states, and if one can identify shock by cooling and sudden constraint with that which is caused by "blows on the neck of the animal so hard that they produce nystagmus and temporary paralysis of the extremities".

Finally, we would like to point out what happens to the erythrocytes during the various conditions described above. Similar to Löwit, we never noticed any significant effects of the various procedures, neither in the experiments with cooling nor shock, on red blood corpuscles. We have not made this test in all cases, since it would have been impossible, especially when it was crucial to make numerous counts in quick succession. But from frequent random tests during the various experiments, we could definitely conclude that the number of erythrocytes was neither increased nor decreased by the measures that affected the leukocytes so strongly.

Regarding the test results per se, we are in general agreement with Löwit. To summarize in brief: we found hypoleukocytosis to be the result of: (1) prolonged cooling, (2) sudden shock, and that both kinds produce a subsequent, low-grade hyperleukocytosis. At the end of this work, we will mention our interpretation of these phenomena, and will test the influences which play a part. Finally, we will investigate whether these phenomena can be explained according to Löwit, on the basis of deterioration, and insufficient influx of white blood corpuscles from the organs producing the blood cells.

Influence of Injection of Nucleic Acid, Proteins, etc., and Organic Extracts on Leukocytosis

Prompted by a case of myxedema which in the clinic for internal medicine was treated with thyroid extract, we wished to prepare extracts of other /387
organs and to test their effect on the normal animal body experimentally. The method of preparing these extracts has been described above and is

generally similar to the prescriptions given by the English researchers Murray, Batter, Fox, Mackenzie, also Laache, Wichmann, Leichtenstern.

The results of injections of organ extracts were already given in our first report. We reported that injection of spleen, thymus and bone marrow extracts produced hypo- and hyperleukocytosis, which extracts from thyroid, liver, kidney and pancreas did not achieve. We chall describe a series of such experiments with figures.

From these experiments, it is apparent that it is possible to produce hypo- and hyperleukocytosis in the animal system by injection of a series of substances. The results of the experiments listed under III in general correspond with those of v. Samson-Himmelstjerna [17], Groth [18], Löwit, Buchner, Römer, Rieder, v. Limbeck, Massart [13], Everard [13], Demoor [13], Werigo [14], Müller [15], Michelson [16] achieved after injecting analogous substances.

Furthermore, the experiments under I. and II. show the manner in which carbolic acid-glycerine extracts prepared from organs affect leukocytosis: extracts of spleen, thymus gland and bone marrow have a considerable effect. Kidney, liver, thyroid and pancreas extracts, however, do not. We had stated this already in our first report, but one point still needs correction. We /395
had mentioned then that we were able to observe hypoleukocytosis as a phenomenon preceding hyperleukocytosis only after injection of spleen extract. Later experiments [see 27, 28, 29, 33] showed that bone marrow and thymus extract also caused this phenomenon. We had probably overlooked this in our first observations, since the last mentioned two extracts have a less intensive effect on leukocytosis than spleen extract. Indeed, this approaches being the most effective substance. In order to avoid any contention about priority, it must be emphasized that the phenomenon of hyperleukocytosis was already observed by Horbaczewski [19, 20] in 1891 when feeding the nuclein of spleen pulp. However, we approached the investigation of the effect of organ extracts on leukocytosis without any knowledge of these observations. Horbaczewski in his investigations was mainly concerned with discovering the

I. SUBCUTANEOUS INJECTION OF LIVER, KIDNEY, THYROID AND PANCREAS EXTRACTS

No.	Injection	Time of day hr min	Leukocyte* count	General Remarks
9.	5 ccm liver extract subcut. in abd. skin	June 10 9:30 am 9:40 am 12:30 pm 4:55 pm 7:20 pm June 11 8:40 am 6:20 pm	 10400 Inj. 9800 9400 9400 10600 10200 9800	Total duration of observation 33 hrs: no change in leukocytes.
10.	Inj. of 6.5 cc liver extract subcut. in abdomen	May 17 10:20 am 10:25 am 12:40 pm 3:20 pm 7:20 pm May 18 8:20 am 12:45 pm 5:30 pm May 19 10:00 am	 11600 Inj. 11000 11800 11000 10600 11000 11400 11200	Total duration of observation 48 hrs. No change of leukocytes.
11.	Inj. of 4 cc kidney extract subcut. in skin of abdomen	May 15 8:50 am 9:00 am 12:20 pm 3:10 pm 6:05 pm 7:10 pm May 16 10:20 am 1:55 pm 6:40 pm May 17 9:05 am	 9800 Inj. 8800 8600 8800 9200 9000 8800 8600 9200	Total duration of observation 48 hrs. No change in leukocytes.

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* The following figures always refer to counts made from the blood of an ear vein.

I. SUBCUTANEOUS INJECTION OF LIVER, KIDNEY, THYROID AND PANCREAS EXTRACTS

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		July 3		
12.		9:45 am	12200	
	Inject 5.5 cc	10:00 am	Inj.	
	kidney extract	12:20 pm	11200	
	subcut. in abd.	2:10 pm	11800	
		4:15 pm	11400	
		6:45 pm	11800	
		July 4		
		8:15 am	12600	Total duration of observation
		2:10 pm	12000	33 hrs: No change
		7:05 pm	12400	
		June 1		
13.		10:00 am	6900	
	Injection of 2 cc	10:15 am	Inj.	
	kidney extract	2:45 pm	7000	
	subcut. in abd.	6:30 pm	7800	
		June 2		Total duration of observation:
		9:00 am	6700	23 hrs. No change.
		June 5		
14.		10:15 am	11400	
	Inj. of 7.5 cc	10:30 am	Inj.	
	pancreas extract	1:05 pm	11000	
	subcutaneous in abdomen	2:55 pm	11800	
		5:45 pm	11000	
		7:30 pm	11200	
		June 6		
		8:40 pm(sic)	11800	Total duration of observation
		1:30 pm	11200	32 hrs. No change.
		6:25 pm	11200	
		June 9		
15.		9:15 am	10400	
	Inj. of 8 cc	9:25 am	Inj.	
	pancreas extract	12:10 pm	10000	
	subcutaneous in	3:00 pm	10600	
	abdominal skin	6:05 pm	10000	
		June 10		
		10:10 am	10800	

I. SUBCUTANEOUS INJECTION OF LIVER, KIDNEY, THYROID AND PANCREAS EXTRACTS

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		4:30 pm	10200	Total duration of observation 50 hrs. No change. /389
		June 11		
		11:05 am	10600	
		June 14		
16.		10:00 am	13200	
	Injection of 8 cc	10:20 am	Inj.	
	of pancreas extr.	1:55 pm	13800	
	subcutaneous into	4:40 pm	12800	
	abdominal skin.	7:05 pm	13200	
		June 15		
		9:05 am	13000	
		1:15 pm	13600	Total duration of observation 49 hrs. No change.
		June 16		
		11:10 am	13200	
		April 4		
17.		10:05 am	10400	
	Injection of 2 cc	10:15 am	Inj.	Thyroid extract was obtained ready-made from
	thyroid extract	12:10 pm	10000	
	subcut. in abd.	5:15 pm	10800	Simon's drugs (Berlin).
		April 5		Total duration of observation 24 hrs. No change.
		10:20 am	10400	
		April 18		
18.		9:30 am	9400	
	Injection of 3 cc	9:50 am	Inj.	
	thyroid extract	2:05 pm	9600	
	subcutaneous	6:20 pm	8800	
	into abd. skin	April 19		
		11:05 am	9400	Total duration of observation 48 hrs. No change.
		April 20		
		10:20 am	9000	
		April 27		
19.		10:20 am	10200	
	Injection of 4 cc	10:35 am	Inj.	
	thyroid extract	1:05 pm	9800	
	subcutaneously	4:10 pm	10400	
	into abd. skin	6:45 pm	10000	

I. SUBCUTANEOUS INJECTION OF LIVER, KIDNEY, THYROID AND PANCREAS EXTRACTS

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		April 28		
		9:30 am	9600	
		5:20 pm	10200	
		April 29		Total duration of observation
		11:05 am	10400	48 hrs. No change.

II. SUBCUTANEOUS INJECTION OF SPLEEN, THYMUS, BONE MARROW EXTRACT

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		April 10		
20.		9:45 am	8200	
	Injection of 2 cc	10:00 am	Inj.	
	spleen extract	1:00 pm	4000 !	
	subcutaneously	4:15 pm	12400	
	into abd. skin	7:20 pm	18200 !	
		April 11		
		9:20 am	16700	
		1:35 pm	17200	First hypo- then hyperleuko-
		6:15 pm	10800	cytosis, after 48 hrs back
		April 12		to normal number of leuko-
		10:05 am	8800	cytes.
		May 1		
21.		8:20 am	9500	/390
	Injection of 1.5	9:05 am	Inj.	
	cc spleen extract	12:00 am	7200 !	
	subcutaneously	5:30 pm	2900 !	
	into abd. skin	May 2		
		8:45 am	18200	
		1:30 pm	16800	First hypo- then hyperleuko-
		6:45 pm	12300	cytosis (triple), after 49
		May 3		hrs. close to normal no. of
		10:15 am	10400	leukocytes.
		3:20 pm	9000	
		May 9		
22.		9:45 am	10400	
	Injection of 8 cc	10:00 am	Inj.	
	spleen extract[1]	1:15 pm	3600 !	
	subcutaneously	8:20 pm	31800 !	
	into abd. skin	May 10		First hypo- then hyperleuko-
		10:10 am	29600	cytosis (up to triple); after

[1] During injection of 8 cc, the animal suffers from collapse, cramps; recovers soon thereafter, being rubbed and wrapped in warm cloths. The injection had probably hit a small vein and the same occurred for Murray during too rapid injection of thyroid extract in man.

II. SUBCUTANEOUS INJECTION OF SPLEEN, THYMUS, BONE MARROW EXTRACT

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		6:30 pm	23400	47 hrs still hyperleukocy-
		May 11		tosis.
		8:45 am	26000	
		May 26		
23.		9:20 am	8600	
	Injection of 6 cc	9:30 am	Inj.	
	spleen extract	1:40 pm	2600 !	
	subcutaneously	4:25 pm	12300	
	into abd. skin	7:20 pm	23800 !	
		May 27		
		10:10 am	17400	
		4:30 pm	12000	First hypo- then hyperleuko-
		May 28		cytosis after 48 hrs normal
		9:30 am	9300	number of leukocytes.
		May 30		
24,		9:35 am	10200	
	Injection of 5.5	9:50 am	Inj.	
	cc subcut. into	11:50 am	8400	
	abd. skin	12:50 pm	5000	
		2:10 pm	4200 !	
		4:35 pm	9100	
		5:55 pm	13600	
		7:05 pm	21600 !	
		May 31		
		10:05 am	20400	
		12:55 pm	17800	
		5:30 pm	11000	
		June 1		First hypo- then hyperleuko-
		8:50 am	10200	cytosis after 31-1/2 hrs
		4:35 pm	11200	normal number of leukocytes.
		June 17		
25.		8:55 am	8800	/391
	Injection of 6.5	9:10 am	Inj.	
	cc spleen extract	12:30 pm	4700	
	subcut. into abd.	2:50 pm	3600 !	
	skin.	6:10 pm	14200	First hypo- then hyperleuko-
		7:35 pm	19800	cytosis which after 24-3/4
				hrs. is still somewhat

II. SUBCUTANEOUS INJECTION OF SPLEEN, THYMUS, BONE MARROW EXTRACT

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		June 18		
		9:55 am	21400 !	stronger than the day before.
		May 2		
26.		11:40 am	9000	
	Injection of 4 cc	11:55 am	Inj.	
	bone-marrow extr.	3:40 pm	8400	
	subcut. into abd.	7:15 pm	19200 !	
		May 13		
		8:10 am	12800	
		2:30 pm	8600	
		May 14		
		10:15 am	9300	Hyperleukocytosis, after 26-1/2 hrs number of leukocytes normal.
		May 24		
27.		9:20 am	8900	
	Injection of 2.5	9:30 am	Inj.	
	cc bone-marrow	12:15 pm	7600 !	
	extr. subcut. into	4:05 pm	14600 !	First minor hypo- then moderate hyperleukocytosis; after
	abd. skin	May 25		
		8:25 am	11000	28 hrs leukocyte count normal.
		1:35 pm	9200	
		June 15		
28.		9:20 am	10400	
	Injection of 6 cc	9:35 am	Inj.	
	bone-marrow extr.	12:10 pm	6200 !	
	subcutaneously	2:30 pm	8600	
	into abd. skin	5:45 pm	17500	
		7:10 pm	20400 !	
		June 16		
		9:40 am	18400	
		1:35 pm	12600	First hypo- then hyperleukocytosis after 47 hrs normal
		June 17		
		8:20 am	10000	leukocyte count.
		July 31		
29.		10:25 am	9200	
	Injection of 6.5	10:35 am	Inj.	

II. SUBCUTANEOUS INJECTION OF SPLEEN, THYMUS, BONE MARROW EXTRACT

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		July 31		
	cc bone-marrow extr.	1:15 pm	4800 !	
		5:20 pm	13500	First hypo- then hyperleukocytosis (by more than double); after 28 hrs leukocyte count not yet normal.
		7:55 pm	21800 !	
		Aug. 1		
		9:30 am		
		2:25 pm	12200	
		May 19		
30.		10:05 am	7100	
	Injection of 2 cc thymus extract subcut. into abd.	10:20 am	Inj.	
		3:50 pm	16400 !	
		6:30 pm	15800	
		May 20		Hyperleukocytosis, after 26 hrs normal leukocyte count.
		12:20 pm	7000 !	
		June 7		
31.		11:20 am	8000	
	Injection of 5 cc thymus extract subcut. into abd.	11:55 am	Inj.	
		6:50 pm	17000	
		8:10 pm	19800 !	
		June 8		
		7:15 am	9200	
		June 12		
32.		9:10 am	9800	
	Injection of 6 cc thymus extract subcut. in abd.	9:30 am	Inj.	
		12:15 pm	7800 !	
		4:30 pm	14600	
		7:10 pm	20200 !	
		June 13		
		8:15 am	12600	
		12:45 pm	9400	First hypo- then hyperleukocytosis; after 27 hrs normal leukocyte count.
		June 14		
		9:25 am	10200	

II. SUBCUTANEOUS INJECTION OF SPLEEN, THYMUS, BONE MARROW EXTRACT

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
33.	Injection of 6 cc thymus extract subcut. in abd.	July 27 9:55 am 10:05 am 1:35 pm 4:20 pm 7:45 pm July 28 9:35 am 4:40 pm	 9200 Inj. 5600 14800 20300 12800 9600	First hypo- then hyperleukocytosis after 30-1/2 hrs normal leukocyte count.

III. SUBCUTANEOUS INJECTION OF OTHER SUBSTANCES

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
34.	Injection of 1 g hemialbumose in 8 cc dist. H20 subcut. in abd.	Sept. 10 9:20 am 9:35 am 12:30 pm 1:45 pm 4:25 pm 7:10 pm Sept. 11 9:30 am	 10400 Inj. 6800 4500 ! 13200 21600 ! 18600	First hypo- then hyperleukocytosis (somewhat more than double).
35.	Injection of 1 g hemialbumose in 10 cc dist. water subcut. in abd.	Dec. 27 9:10 am 9:20 am 12:55 pm 6:10 pm Dec. 28 10:15 am 5:35 pm	 8800 Inj. 4200 ! 17300 20800 ! 10200	First hypo- then hyperleukocytosis (increase by 2-1/2; normal leukocyte count after 32-1/4 hrs).
36.	Injection of 0.2 g nucleic acid in 3.2 cc dist. water intraperitoneal	Dec. 15 1:50 pm 2:00 pm 6:15 pm Dec. 16 9:00 am Dec. 17 12:10 pm	 9800 Inj. 11200 19600 ! 8600	
37.	Injection of 0.4 g nucleic acid in 6.4 cc dist. water intraperitoneal	Dec. 15 2:30 pm 2:40 pm 6:00 pm Dec. 16 9:30 am 6:30 pm Dec. 17 12:10 pm Dec. 18 9:30 am	 10400 Inj. 6600 ! 10400 21200 ! 17800 9300	Animal seems ill. Animal still ill. This animal having received twice the dose of the previous animal shows definite hypoleukocytosis and hyperleukocytosis only after 28 hrs normal count after 64 hrs.

III. SUBCUTANEOUS INJECTION OF OTHER SUBSTANCES

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
		Dec. 15		
38.		2:45 pm	10800	
	Injection of .5 g	2:55 pm	Inj.	
	nucleic acid in	6:50 pm	8800	Animal quite listless,
	8.0 cc dist. water	Dec. 16		apparently ill.
	intraperitoneal	9:50 am	5600 !	
		5:35 pm	6200 !	Even after 26-1/2 hrs hypo-
		Dec. 17		leukocytosis; only after about
		11:40 am	10200	70 hrs moderate hyperleuko-
		Dec. 18		cytosis.
		12:45 pm	18400	Animal somewhat livelier, but
		Dec. 20		still ill.
		morning	—	Animal dead; dissection shows
				massive swelling of the spleen.
		April 14		
39.		9:40 am	10400	
	Injection of 1 cc	10:00 am	Inj.	
	aqueous suspension	1:20 pm	4300 !	First hypo- then hyperleuko-
	of Staph. aureus	6:35 pm	19800	cytosis; after 48 hrs normal
	intraperitoneal	April 15		number of leukocytes;
		9:10 am	24600 !	Site of injection greatly
		4:30 pm	13500	swollen.
		April 16		
		10:20 am	11000	
		April 20		
40.		8:55 am	8600	
	Injection of 1.5	9:15 am	Inj.	
	cc of same sus-	12:05 pm	3200 !	
	pension intra-	6:40 pm	10800	
	peritoneal	April 21		
		9:10 am	27400 !	First hypo- then hyperleuko-
		1:40 pm	21800	cytosis (more than triple)
		7:35 pm	16800	after 49 hrs not quite ini-
		April 22		tial count.
		10:25 am	10200	

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III. SUBCUTANEOUS INJECTION OF OTHER SUBSTANCES

No.	Injection	Time of day hr min	Leukocyte count	General Remarks
41.		April 2		
		10:10 am	9600	
	Injection of 1 cc	10:30 am	Inj.	First hypo- then hyperleuko-
	suspension of Bac.	1:10 pm	4000 !	cytosis, after 48 1/2 hrs
	pyocaneus cul-	5:55 pm	10200	normal number of leukocytes.
	ture, ca. 8 days	April 8		
	old; intraperi-	8:35 am	23800 !	Animal very exhausted, some
	toneal	4:20 pm	17600	swelling.
		April 9		
		11:10 am	9200	
42.		April 7		
		9:25 am	10200	Even after 6 1/2 hrs hypo-
	Injection of 2 cc	9:45 am	inj.	leukocytosis.
	of same suspen-	12:30 pm	3600 !	
	sion intraperi-	4:10	4100	
	toneal	7:05	Death	Animal dead; dissection showed strong swelling of peritoneum, spleen en- larged.

relationship between excretion of xanthin bases and hyperleukocytosis, not the nature of the latter itself. This makes the viewpoints from which we conducted our experiments entirely different from his. The co-author is at this time engaged in studying this relationship and has briefly reported on this recently in the Deutsche Medizinische Wochenschrift.

At the end of the work, we will discuss how we explain the action of the various substances on leukocytosis and specifically the differences of the individual organ extracts.

Now we come to a new series of investigations carried out in order to test Schulz's concept of the nature of leukocytosis.

Critique of Schulz's Theory

Schulz's theory has already been mentioned in our introduction. At this point, we consider a more detailed critique necessary, and for this purpose we first must discuss some of his experimental results.

On p. 274 of his work Schulz relates that he conducted a control operation on a dog in order to establish if the increased number of leukocytes found after extirpation of the spleen was caused by the extirpation as such, or only by the surgical wound. He made a 4 cm incision along the linea alba to the peritoneum in a healthy young dog, without removing the spleen he sewed up the various layers, and after 4 days the wound had healed aseptically. Schulz reports during these four days, quite an enormous hyperleukocytosis (e.g., 54300, 22-1/2 hrs. after the operation, i.e., an increase of 370%); furthermore, this hyperleukocytosis set in immediately after the operation: 62% increase after half an hour, 234% after another 4-1/2 hours. /396

These results are very surprising, especially since they are in direct opposition to all previous observations. The increase immediately after the operation cannot be termed posthemorrhagic, since the animal could hardly

have lost much blood. On the other hand, Schulz does not mention any special precautions to warm the animal, which certainly must have been constrained. Therefore, the exact opposite was to be expected after the operation, namely hypoleukocytosis due to cooling and restraint, Löwit's leukopenia which we have discussed in detail above. Even if Schulz had actually taken suitable precautions to avoid cooling, his findings would still be contrary to ours, for we were never able to claim hyperleukocytosis due to the operational wound alone, avoiding cooling. It also seems very peculiar that Schulz's experiment show 54,300 leukocytes 23 hours after the operation, a value which, in general, is only obtained by the strongest injections, especially when Schulz expressly states that there were no signs of peritonitis during the healing process of a wound designed to achieve hyperleukocytosis. The results of this observation are not in harmony with numerous others: Tarchanoff's test which revealed no hyperleukocytosis by inflicting an abdominal wound on a dog and closing it immediately; also our experiments described above, showing that no hyperleukocytosis occurs when wounds exposing the jugular and /397
femoral vein are closed after 2-3 hours when, during this time, hypoleukocytosis due to cooling is avoided. Finally, the experiments of Emelianow [22] are to be considered, who had results similar to ours.

The numerical values found by Schulz in spleenectomized animals also contradict all other reports so far. He claims hyperleukocytosis after spleen extirpation in a dog only during the first six days; in a rabbit — during the first 10 days after the operation. All experimental investigations published till now on this subject report that the increase of white blood corpuscles after spleen extirpation lasted at least 1-1/2 - 2 months. Zesas [23] reports that he found a significant increase of leukocytes mainly large ones in the blood of a rabbit 4 weeks after spleen extirpation. Pean claims hyperleukocytosis in one case several weeks after the operation; in a second case — even after 3-1/2 months. Also, Crede [24] on the basis of his findings comes to the conclusion that spleen extirpation produces hyperleukocytosis for several weeks.

From these investigations, it is apparent that hyperleukocytosis following spleen extirpation is of much longer duration than Schulz describes and is hardly caused by infection. Schulz considers this the main reason for the phenomenon, but at the same time he is astonished that this occurs at all, since one of the blood-producing organs is eliminated.

Now we come to the main point of Schulz' work, i.e., to the statement made by him that the numerical differences found in hypo- and hyperleukocytosis are only due to a varying distribution of leukocytes in the vascular system. He bases this theory on results of certain experimental observations in which he produced hypo- and hyperleukocytosis by injecting bacteria proteins and cultures of bacteria, reaching the conclusion that if the former exist in the peripheral vessels, the latter must exist in the central ones, and vice versa. We shall give here some of Schulz's values (see Table p. 31).

First, we would like to present some critical remarks about some of the findings. Experiment No. 20 cannot be considered as having been performed with a normal animal since a leukocyte count of almost 20,000 must by all /398
present results be considered pathological. In experiment 22, it is notable that two different counts from the same vein (splenic vein), presumably made simultaneously, amount to 11,600 and the other — more than twice that much, 28,000. Comparing experiments 19-24, it is immediately apparent that in the first two the difference of leukocytes in the vessels is much larger that in experiments 22-24, noting only parenthetically that before the count the animals had been killed by a blow on the neck. Comparing the individual experiments, it becomes apparent how much the results differ among themselves. One time Schulz finds, for instance, in the portal vein 2-1/2 times more leukocytes that in the ear vein (experiment 20); another time, hardly twice the amount (experiment 21); a third time, the same amount in both veins (experiment 24). One time the inferior vena cava contains 2/3 as many leuko- /399
cytes as the ear vein (experiment 19); in another experiment (21), the relationship is 9,900:23,100; in a third, 8,800:5,800. All in all, experiments 19-24 hardly contain two values which correspond with one another relatively speaking.

	normal animals						animals with injections				
	Getödtet. 19.	20.	21.	22.	23.	24.	52.	53.	54.	55.	56.
R. ear vein	11950	19750	9900	8800	11600	9250	66900	5600	79900	74400	58800
L. ear vein	.	.	.	8900	10550	.	.	3300	.	11900	.
stomach vein	.	13900	.	.	.	.	.	21000	.	.	.
kidney vein	.	36400	.	11600	13300	.	.	.	8800	.	.
				28000	15600						
V. mesent. sup.	17200	.	.	3400	9300	.	15000	.	.	.	.
V. mesent. inf.	.	.	.	.	15100	.	.	.	.	.	.
V. portae	.	49800	18400	.	.	9400	46100	27300	10100	.	52700
V. hepatica	.	21800	14500	.	.	7600	23500	18800	22200	.	34300
V. subcl. sinistra	.	18900	.	.	.	.	.	.	.	.	.
Aorta abdom.	.	13100	.	5000	12500	1900	.	.	8600	.	.
V. femoralis	.	.	13400	.	.	.	.	6400	.	.	.
spleen artery	.	.	.	3400	.	.	.	.	.	.	.
V. cava inf.	7200	.	23100	5800	.	.	15900	26600	.	.	7700
Ventr. dexter	.	.	3600	.	.	.	.	.	.	.	.
Ventr. sinister	.	.	7400	.	.	.	9800	3500	4900	.	.
small intestine artery	.	.	.	.	.	7200	.	.	.	.	.
small intestine vein	.	.	.	.	.	7100	.	.	.	.	.
						8500					
V. jug. sin.	.	.	.	.	.	.	6900	.	.	.	.
V. cava sup.	.	.	.	.	.	.	16000	.	.	.	.
V. renalis	.	.	.	.	.	.	.	20100	.	.	.
Art. carotis	.	.	.	.	.	.	.	.	9400	.	.
large intestine vein	.	.	.	.	.	.	.	.	.	.	30300

This is even more apparent in experiments 52-56. Firstly, there are several values which can hardly be interpreted. In experiment 54, for instance, the value of 79,900 which Schulz found in the ear vein was 6,900 1-1/4 hours before that (8,600, initially). Such a rapid change from hypo- to hyperleukocytosis to our knowledge has never been observed. In experiment 55, after injecting 2.0 sterilized culture of Streptococcus pyogenes, Schulz finds 14,600 two hours later in the left ear vein, while at the same time — 71,800 leukocytes in the right ear vein. Due to an unsuccessful injection attempt, some of the liquid had been placed under the skin near the vein of the right ear. After two hours, the same relationship still held: left, 11,900; right, 74400. Also, in these experiments the values do not agree: thus once (experiment 52) hyperleukocytosis is claimed in the ear vein while the portal vein carries only one third of the leukocytes. Another time,

almost the same number was found (experiment 56); a third time — one-eighth (experiment 54). But, here again, we have to emphasize that the leukocyte count from the different vessels for almost all animals was done after they had been killed beforehand.

Comparing all the experiments mentioned, two things stand out: first, the great differences in the various vessels of the same animal. Also, the fact that animals with enormous hyperleukocytosis in the ear vein showed hardly any in the other vessels, in contrast to the animal which showed a definite hyperleukocytosis in the ear vein and also an increase of white blood corpuscles in all other vessels. On the basis of these results, Schulz established his theory on the nature of leukocytes. But this seemed to us hardly plausible, since on further scrutiny we found too many contradictions and inexplicable results, but also because a review of the literature on leukocytosis did not show such results. Only Römer, and later on Rieder, have observed that blood becomes richer in leukocytes toward the periphery, /400 but they had not arrived at a theory analogous to Schulz's. In order to come to our own conclusion about Schulz's observations, we have repeated his experiments. We investigated the various vessels in normal, as well as in injected rabbits in states of hypo- and hyperleukocytosis. However, we observed several precautionary measures which Schulz seemingly had ignored, which probably led him to results so different from ours. First, we ascertained in the manner described above that the animals did not cool during the experiment, then also — and this is of greatest importance as is apparent from the following — we never killed the animal beforehand, but always tested the living vessels. Therefore, we had to limit ourselves to counting from fewer vessels than Schulz did in order to arrive at correct results. We also consider this precaution necessary, since counting from so many vessels causes the particular animal to lose much blood affecting the experimental results.

For the sake of clarity, we will present our findings in tabular form, similar to Schulz's presentation. From these experimental results, we can briefly draw the following conclusions:

A. COUNTS IN A STATE OF HYPERLEUKOCYTOSIS

No.	Injection	Time of day	Site of count	Leukocyte number/1 cmm	General remarks
		June 23			
43.	Injection of 6 cc spleen extract subcutaneous into abdomen.	3:00 pm	Ear vein	7200	
		June 24			
		9:00 am	ditto	24000	
		2:30 pm	ditto	14500	
		2:40 pm	Fem.art.	7800 8400	
		2:50 pm	ditto	7600 8800	
		3:00 pm	ditto	8400	
		3:05 pm	peripheral calf vein	13600 12800	
		3:10 pm	Fem.vein	10200	Animal collapses, dies.
		3:40 pm	Rt. heart	19400	
			Lft.heart	64000 !	
		July 5			
44.	Injection 3 cc spleen extract into abdominal skin subcutaneously	1:00 pm	Ear vein	9400	
		July 6			
		10:00 am	ditto	17600	
		11:00 am			
		5:30 pm	Ear vein	19200	
		5:35 pm	peripheral calf vein	17800	
		5:38 pm	Fem.vein	10400 11000	
		5:43 pm	Fem.art.l.	10600	
		5:47 pm	Fem.art.r.	10200	
		5:52 pm	V.cava inf.	7800 8600	
		5:55 pm	Aorta abd.	7600 6800	
		5:57 pm	Ear vein	20400	
		6:02 pm	R.Ventri.	8200	
		6:03 pm	L.Ventri.	7400	
		July 10			
45.	Injection of 5cc bone-marrow extract subcutaneously into abdominal skin.	6:20 pm	Ear vein	10200	
		6:25 pm			
		July 11			
		10:55 am	ditto	21400	
		11:20 am	ditto	20800	
		11:25 am	Fem.vein l.	17600	
		11:27 am	Fem.vein r.	17000	
		11:31 am	Fem.art.	16200	

A. COUNTS IN A STATE OF HYPERLEUKOCYTOSIS

No.	Injection	Time of day	Site of count	Leukocyte number/1 cmm	General remarks
		July 11			
45.	Injection of 5 cc bone-marrow extract subcutaneously into abdominal skin.	11:36 am	V.ing.ext.	17800	
		11:40 am	Aorta abd.	11600	
		11:43 am	V. cava inf.	12800	
		11:44 am	L.Ventri.	9800	
		11:45 am	R.Ventri.	10800	/401
		July 10			
46.	Injection of 3 cc Bacterium pyocyaneus protein subcutaneously into abdominal skin.	6:40 pm	Ear vein	10600	
		July 17			
		10:55 am	ditto	31600	
		11:20 am	Fem.vein	25200	
		11:25 am	Fem.art.	23000	Animal dies.
		11:40 am	V. renalis	18600	
		11:45 am	V. hepatica	32000	The tremendous increase in the number of leukocytes in the central vessels after death is notable (cf Schulz).
		11:52 am	V. lienalis	44600	
		11:54 am	V.cava inf.	25800	
		11:56 am	R.Ventri.	28600	
		11:57 am	L.Ventri.	39400	

B. COUNTS IN A STATE OF HYPOLEUKOCYTOSIS

No.	Injection	Time of day	Site of count	Leukocyte number/1 cmm	General remarks
		June 29			
47.		1:00 pm	Ear vein	8200	
	Injection of 5 cc	1:25 pm			
	spleen extract	4:00 pm	ditto	5600	
	subcutaneously			5200	
	in the abdominal	4:05 pm	Fem.art.	4200	
	skin.			3400	
		4:12 pm	Fem.vein	4800	
				4000	
		4:15 pm	V.cava inf	1800	
		4:17 pm	Aorta abd.	2200	
				2000	
		4:20 pm	R.Ventri.	4600	
		4:21 pm	L.ventri.	2200	
		June 30			
48.		2:00 pm	Ear vein	10200	
	Injection of 3 cc	2:05 pm			
	spleen extract	July 1			
	subcutaneously	8:00 am	ditto	23600	
	into abdominal	12:30 pm			
	skin.	4:00 pm	ditto	3400	
		4:10 pm	Fem.art.	2600	
				2000	
		4:15 pm	Vena fem.	2600	
				2200	
		4:18 pm	Aorta abd.	1800	
		4:21 pm	Vena cava	2200	
				2000	
		4:22 pm	R.Ventri.	1600	
		4:24 pm	L.Ventri.	2400	
		July 4			
49.	Injection of 3 cc	10:00 am	Ear vein	9800	
	Bacterium pyo-	12:40 pm	ditto	3000	
	cyaneous protein	12:45 pm	Fem.vein	2000	
	subcutaneously	12:50 pm	Fem.art.	1600	
	into abdominal	12:54 pm	V.lienalis	8200 !	The high count
	skin.	12:57 pm	V.cava inf.	1400	from the lienalis
		12:59 pm	Aorta abd.	1600	vein is remarkable.
		1:03 pm	R.Ventri.	1000	
		1:05 pm	L.Ventri.	1400	

/402

No.	Injection	Time of day	Site of count	Leukocyte number/1 cmm	General remarks
		June 27			
50.	—	1:00 pm	Ear vein	11000	—
		1:20 pm	Peripheral	10800	
		1:35 pm	Fem.vein	7800 6800	
		1:45 pm	Ear vein	12200	
		1:50 pm	Fem.art.	6200 6400	
		June 28			
		10:10 am	Ear vein	12800	
		July 12			
51.	—	11:40 am	Ear vein	10800	—
		11:45 am	Fem.art.	7000 6400	
		11:55 am	Fem.vein	7400 7200	
		12:00 pm	V. cava.	2800	
		12:04 pm	Art.renalis	3600 2800	
		12:06 pm	L.Ventri.	3000 2800	
		12:09 pm	R.Ventri.	2600	
		July 18			
52.	—	12:10 pm	Ear vein	7200	
		12:20 pm	Fem.art.	4200	
		12:50 pm	Fem.vein	11000 !	Death.
		12:55 pm	V. hepatica	56000 !	The tremendous increase in the number of leukocytes in the central blood vessels after death is remarkable (cf. Schulz).
		1:05 pm	V. renalis.	85000 !	
		1:10 pm	L.Ventri.	12000 !	
		1:12 pm	R.Ventri.	29600 !	

The distribution of white blood corpuscles in the blood of an animal organism differs, but follows a constant relationship. In all circumstances, normal, as well as artificial, hypo- and hyperleukocytosis, the peripheral vessels always contain a larger amount of leukocytes than the central ones. There is a proportionately high number of leukocytes in the central vessels at the time we claim there is an increase in the peripheral vessels. The same thing is observed in reduced artificial hypoleukocytosis for the entire blood stream, in all vessels accessible to us for counting. According to our /404 test results, we must definitely reject Schulz's concept of the nature of leukocytosis; we were never able to observe, as he did, during existing hyperleukocytosis in the peripheral vessels a corresponding reduction in the central ones and vice versa, i.e., hypoleukocytosis in the former compensated by an increase of white blood cells in the latter.

It is not necessary here to examine what caused the differences between our test results and those of Schulz. This is particularly difficult, since Schulz's work does not sufficiently describe the test arrangement. However, the importance of the test arrangement and the numerous and considerable errors which may arise has already been shown. We only wish to indicate a few points which perhaps explain the differences between the results of Schulz and our results: uniform warming of the experimental animal, i.e., avoiding hypoleukocytosis by cooling (Löwit's leukopenia), always using new, unused animals, taking blood from relatively few vessels in order to avoid errors due to excessive blood loss; and especially not killing the animals before the last count. The importance of this last point becomes apparent from Experiment 52 /405 where a series of counts were made after sudden death, i.e., death caused by a blow on the neck. In this case, however, we received completely different results from all of our other experiments — that is, very high numbers of leukocytes in the central vessels which were not found anywhere else, e.g., 85,000 in the renal vein, 29,600 in the right ventricle, etc. But in this case one cannot speak of a legitimate distribution of leukocytes in the blood stream, and we are dealing with such undeterminable influences as death upon the distribution of the white blood cells. In my estimation, we cannot reach any conclusions from these post-mortem counts. But in most of the

	normal animals			animals with injection						
				Hyperleukocytose.				Hypoleukocytose.		
R. ear vein.	I. 11100 II. 12200	10800	7200	14500	I. 19200 II. 20400	21400	31600	5600 5200	3400	3000
L. ear vein	III. 12800	.	.	.	.	.	.	.	.	.
Peripher. lower leg vein	10800	.	.	13600	17800	.	.	.	.	.
R. V. femoralis	7800 6800	7400 7200	.	10200	10400 11000	17000	25200	4800 4000	2600 2200	2000
R. Art. femoralis	6200 6400	7000 6400	4200	7800 8800	10200	16200	23000	3400 4200	2600 2000	1600
Art. renalis . . .	.	3600 2800	.	.	.	.	.	.	.	.
Vena cava inf. .	.	2800	.	.	7800 8600	12800	25800 !	1800	2280 2000	1400
Aorta abdom. . .	.	.	.	.	7600 6800	11600	.	2200 2000	1800	1600
Vena lienalis . .	.	.	.	.	.	.	44600 !	.	.	8200
R. ventricle	.	2600	.	19400	8200	10800	28600 !	4600	1600	1000
L. ventricle	.	3000 2800	.	64000 !	7400	9800	39400 !	2200	2400	1400
L. V. femoralis .	.	.	.	.	.	17600	.	.	.	.
L. Art. femoralis.	.	.	.	10600	.	.	.	.	.	.
V. jug. ext. sin. .	.	.	.	.	.	17800	.	.	.	.
V. renalis	.	.	.	.	.	.	18600 !	.	.	.
V. hepatica . . .	.	.	.	.	.	.	32000 !	.	.	.

experiments Schulz tested the vessels after the animal had been killed by a blow on the neck. These brief remarks may suffice to explain to some degree the difference between Schulz's results and ours. Only at the end of our work can we elaborate on the latter, when we will attempt to explain the nature of hypo- and hyperleukocytosis.

Löwit's Leukolysis

From the experiments mentioned in the previous chapters, we can see that we always observed hypoleukocytosis preceding hyperleukocytosis from our numerous counts. Thus, the supposition that Löwit's theory was valid emerged: "Leukocytosis (hyper-) constitutes a temporary increase above normal of leukocytes in all of the blood preceded by a reduction caused by various factors, including increased influx of immature leukocytic elements from

blood-forming organs. Leukocytosis probably arises in all cases of the destruction or disappearance of leukocytes in the blood."

In the following series of experiments, we tried to discover this relationship between hypo- and hyperleukocytosis, which according to Löwit, exists by conducting the experiments in an analogous manner to his. Individual modifications of test arrangements which we considered suitable have been described at the beginning. We warmed the test animal uniformly, and the animals used in these experiments were almost always fresh, or when previously tested animals were to be used for new experiments, it was only after the change in leukocytosis was completely normal again, i.e., we could determine the original normal number of white blood corpuscles. First, we re-examined Löwit's experiments with some of the substances used by him, specifically, hemialbumose. Later on, we used primarily those organ extracts which, according to our tests, were equivalent to these substances with regard to their effect on leukocytosis. First of all, we shall give our experimental data.

It is clearly apparent from these and earlier experiments that every hyperleukocytosis is preceded by a more or less considerable hypoleukocytosis and only in single points do our test results differ from Löwit's. In connection with our presentation in the previous chapter, we need to qualify, above all, Löwit's statement that hypoleukocytosis produced by injections is equal in all blood vessel regions. It can, however, be claimed to exist both in the peripheral and the central vessels, thereby supporting Löwit's contention that during the state of hypoleukocytosis, there is no compensatory hyperleukocytosis to be found in any vessel. Regarding the speed of development of hypoleukocytosis following the injections, it is Löwit's opinion that /412 the highest degree is already reached after seconds. The test results of the students of Alexander Schmidt support this opinion, which indicated a minimum number of leukocytes already at the time of the fibrinase injection (fibrin enzyme), cultures of putrefaction and other substances. We were never able to achieve such a rapid production of hypoleukocytosis after the injection of our substances; the maximum was reached only several minutes after injection.

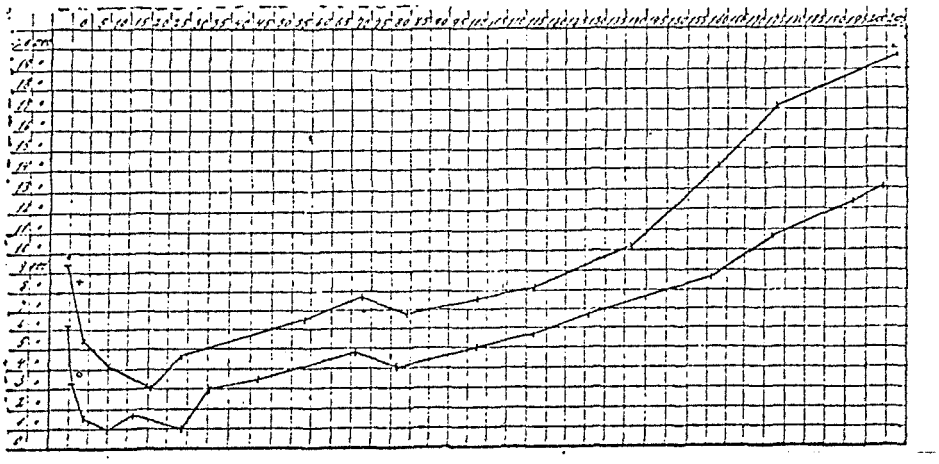
No.	Injection	Time of day	Leukocyte*	General remarks
53.		September 10 12:25 pm	10200 (Ear vein) (5,350,000 Er.)	
	Injection of 6 cc 8% solution of hemialbumose into left external jugular vein.	12:32.45 pm 12:33.10 pm 12:33.15 pm 12:41.22 pm 12:46.35 pm 12:58.— pm 1:17.25 pm 1:33.— pm	Inj. 1400 ! (5,300,000 Er.) 3400 3000 4200 5800 5400	Strong hypoleukocytosis after 25 sec. The animal is used for other purposes.
54.		September 11 2:—.— pm	8200	
	Injection of 6 cc of same substance into left external jugular vein.	2:01.30 pm 2:01.45 pm 2:05.28 pm 2:05.40 pm 2:12.55 pm 2:15.25 pm 2:26.22 pm 2:28.08 pm 2:36.28 pm	Inj. 6600 2600 Fem.art. 3400 3000 Fem.art. 1000 ! Fem.art. 2600 2400 6200	 Maximum hypoleukocytosis observed in the artery after 14 minutes. The animal dies 1/2 hour after the experiment.
55.		September 18 1:20.— pm	8200	/407
	Injection of the same substance into the left external jugular vein.	1:25.02 pm 1:28.50 pm 1:29.30 pm 1:34.05 pm	Inj. 3400 1800 600 !	Maximum hypoleukocytosis after 9 min. Animal used for other purposes.
56.		September 23 1:20.— pm	7600	
	Injection of 6 cc of same substance into left external jugular vein.	1:24.10 pm 1:28.40 pm 1:31.30 pm 2:02.— pm	Inj. 4200 ! 1800 3200	Maximum hypoleukocytosis after approx. 7-1/4 min.

*If no other data are given, the number of leukocytes refers to the counts made from the femoral vein.

No.	Injection	Time of day	Leukocyte	General remarks
		September 23		
56.		2:08.32 pm	5800	
		2:10.— pm	6600	Animal used for microscopic examination.
		September 14		
57.		1:50.— pm	7200	
	Injection of 8 cc spleen extract into left external jugular vein.	1:54.20 pm	Inj.	
		1:57.40 pm	600 !	
		2:—.50 pm	1200	
		2:03.50 pm	1200	
		2:07.12 pm	Ear vein 11200	
		2:07.30 pm	800	
		2:14.10 pm	2800	These experiments show that the number of leukocytes in the various vessels varies during initial development of hypoleukocytosis, but soon the normal relationship between peripheral and central vessels develops. The animal is used for microscopic investigations.
		2:18.15 pm	Ear vein 1800	
		2:21.40 pm	Ear vein 1200	
		2:22.08 pm	3200	
		2:30.30 pm	Ear vein 800	
		2:30.48 pm	2800	
		2:35.25 pm	Ear vein 1800	
		2:36.20 pm	3200	
		2:45.58 pm	Ear vein 3800	
		2:46.35 pm	4200	
		3:06.05 pm	Ear vein 13600	
		3:06.30 pm	4800	
		September 16		
58.		1:35.— pm	9800	
			Ear vein 14600	Maximum hypoleukocytosis after 4 minutes.
	Injection of 6 cc spleen extract into left external jugular vein.	1:38.02 pm	Inj.	
		1:40.10 pm	5400	
		1:42.— pm	V. jug. 600 !	
		1:45.28 pm	Atr. dextr. 1000	
		1:47.55 pm	Ventr.dextr. 600	Animal dies.
		September 23.		
59.		9:—.— am	Ear vein 10200	
	Injection of 6 cc spleen extract into external jugular vein.	9:15.— am	Inj.	Incision is stitched, the animal is put into the cage wrapped in warm clothes. After 2-1/2 hrs., hyperleukosis has doubled. /408
		10:45.— am	Ear vein 8800	
		11:50.— am	Ear vein 21000	
		12:30.— pm	Ear vein 25600	
		1:47.— pm	Ear vein 27400	

No.	Injection	Time of day	Leukocyte	General remarks
59.		September 23		Animal is used for microscopic examinations.
60.	Injection of 8 cc spleen extract into external jugular vein.	September 25 1:40.— pm 1:44.— pm 1:44.45 pm 1:46.40 pm 1:48.50 pm 1:51.— pm 1:52.45 pm	15200 ! Inj. 5800 6800 5400 3000 2600	Animal is killed for purpose of microscopic examination.
61.	Injection of 8 cc spleen extract into left external jugular vein.	September 27 1:43.35 pm 2:13.05 pm 2:13.50 pm 2:15.30 pm 2:17.45 pm 2:21.10 pm 2:24.10 pm 2:24.30 pm	Ear vein 9500 V. fem. 6200 Inj. V. fem. 3400 Ear vein 5800 V. fem. 1800 Ear vein 4200 Ear vein 4600 V. fem. 1000 !	Maximum hypoleukocytosis after 11 min. 25 sec.

/409



Injection of 8 cc spleen extract into jugular vein.

Note: The ordinate shows the number of leukocytes; the abscissa gives the minutes. The marks on the curves show the times of leukocyte count.

The upper curve refers to the ear vein; the lower to the femoral vein.

61.

September 27	
2:29.40 pm	Ear vein 3200
2:30.05 pm	V. fem. 1600
2:36.15 pm	Ear vein 4600
2:36.50 pm	V. fem. 1200
2:49.15 pm	Ear vein 6600
2:50.35 pm	V. fem. 3000
3:04.45 pm	Ear vein 6400
3:05.20 pm	V. fem. 3400
3:23.30 pm	Ear vein 7600
3:24.20 pm	V. fem. 4800
3:35.50 pm	Ear vein 6900
3:36.25 pm	V. fem. 4200
3:47.20 pm	Ear vein 6600
3:47.50 pm	V. fem. 4800
4:—.10 pm	Ear vein 7400
4:—.35 pm	V. fem. 5000
4:14.30 pm	Ear vein 8200
4:14.55 pm	V. fem. 5600
4:27.40 pm	Ear vein 10600

No.	Injection	Time of day	Leukocyte	General remarks
		September 27		
61.		4:28.40 pm	V. fem. 7000	
		4:43.55 pm	Ear vein 10000	
		4:44.50 pm	V. fem. 6400	
		5:01.— pm	Ear vein 14200	
		5:01.25 pm	V. fem. 8600	
		5:14.30 pm	Ear vein 17200	
		5:14.55 pm	V. fem. 12800	After 3 hours, 34 min. hypoleukocytosis is more than double. Animal used for microscopic tests.
		5:35.25 pm	V. fem. 12400	
		5:41.50 pm	Ear vein 19000	
		5:47.05 pm	V. fem. 14800	
		November 16		
62.		5:—.— pm	7600	
	Injection of 8 cc spleen extract into left external jugular vein.	5:10.35 pm		
		5:13.10 pm	1600	After 3 min. 55 sec., masimum hypoleukocytosis.
		5:14.30 pm	1000 !	
		5:16.28 pm	1400	
		5:18.45 pm	1800	
		5:21.20 pm	1400	
		5:25.23 pm	2200	
		5:30.35 pm	2800	
		5:37.20 pm	3000	
		5:49.50 pm	3600	
		6:—.10 pm	4200	
		6:14.30 pm	5000	
		6:26.— pm	6400	
		November 17		
		9:30.— am	Ear vein 18800	
		October 22		
63.		5:40.— pm	7200	
	Injection of 8 cc bone marrow extract into left external jugular vein.	5:49.04 pm	Inj.	
		5:50.06 pm	5200	Maximum hypoleukocytosis after 7 min. 20 sec. Note: it is not as strong as with spleen extract. Animal used for microscopic investigation.
		5:51.07 pm	3600	
		5:53.53 pm	2600	
		5:56.23 pm	1800 !	
		6:02.10 pm	2200	

No.	Injection	Time of day	Leukocyte	General remarks
		November 1		
64.		5:02.— pm	12600 !	/410
	Injection of 8 cc bone marrow extract into left external jugular vein.	5:04.05 pm	Inj.	
		5:06.30 pm	4200	Maximum hypoleukocytosis after 16 min. 15 sec., again not as strong as after spleen extract.
		5:08.— pm	4200	
		5:10.04 pm	2800	
		5:14.30 pm	2200	
		5:17.25 pm	1800 !	
		5:20.20 pm	2800	
		5:28.— pm	3600	Incision is stitched, animal is put into cage wrapped in warm clothes.
		5:37.35 pm	5400	
		5:49.50 pm	8200	
		5:57.06 pm	9400	
		November 2		
		11:30.— am	Ear vein 21400	Animal is lively, no swelling at site of injection.
		November 19		
65.		2:09.— pm	6200	
	Injection of 0.2 g nucleic acid in 3.2 cc distilled water into left external jugular vein.	2:10.45 pm	Inj.	
		2:12.05 pm	5400	
		2:13.30 pm	3600	
		2:15.05 pm	2600	
		2:21.30 pm	2800	Maximum hypoleukocytosis after about 20 min.
		2:26.30 pm	1800	
		2:30.35 pm	600 !	
		2:35.25 pm	1200	
		2:44.20 pm	1000	
		2:55.— pm	1600	
		3:07.30 pm	2000	
		3:33.— pm	3600	Even after 20 hours, strong hypoleukocytosis. Animal gives a very sick impression, is killed for purposes of microscopic tests.
		3:56.— pm	3200	
		6:06.45 pm	Ear vein 4200	
		December 20		
		12:—.— am	Ear vein 3600	
		September 21		
66.		1:—.— pm	Ear vein 11600	
	Injection of 4 cc pancreas extract into left external jugular vein.	1:05.30 pm	Inj.	
		1:05.45 pm	11200	
		1:07.15 pm	12600	
		1:09.30 pm	12600	

No.	Injection	Time of day	Leukocyte	General remarks
		September 21		
66.		1:11.30 pm	12000	
		1:13.20 pm	11200	
		1:14.45 pm	11400	
	Repeat injection of 4 cc pancreas extract into same vein.	1:29.— pm		The experiment shows that intravenous injections of pancreas extract do not produce any change in leukocytosis.
		1:29.45 pm	10600	
		1:31.20 pm	9800	
		1:33.15 pm	11000	
		1:34.55 pm	10600	
		1:38.— pm	10200	
		1:40.17 pm	10400	
		1:43.— pm	9600	Animal is used for microscopic investigations.
		September 19		
67.		1:—.— pm	8400	
	Injection of 4 cc kidney extract into left external jugular vein.	1:10.— pm	Inj.	Immediately after the injection, the animal has strong dyspnea and cramps; recovers after about 6-7 min. /411
	Once more, injection of 4 cc kidney extract into the same vein.	1:20.— pm	Inj.	Once again, violent convulsions and dyspnea; ded after about 1 min.
		September 19		
68.		1:24.— pm	8200	
	Injection of 4 cc kidney extract into left external jugular vein.	1:25.02 pm	Inj.	
		1:25.17 pm	9800	
		1:26.55 pm		Animal dies with strong convulsions.
		September 26		
69.		2:03.— pm	16600	Animal was used before.
	Injection of 8 cc kidney extract into left external jugular vein.	2:05.15 pm	Inj.	
		2:06.— pm	15400	After about 15 min. minor hypoleukocytosis which is probably due to the generally bad condition of the animal.
		2:10.05 pm	13200	

No.	Injection	Time of day	Leukocyte	General remarks
		September 26		
69.		2:12.40 pm	14200	Animal somewhat dyspnoic.
		2:15.35 pm	12800	
		2:20.10 pm	13800	
		2:30.— pm		Animal dies in convulsions.

We do not wish to attach any great significance to this difference in test results. On the one hand, the speed of the effect depends on the varying nature of the substances introduced. On the other hand, our experimental procedure differed from Löwit's in that he took the blood for his counts from the peripheral part of the inquinal vein — i.e., the carotid — however, we took it from the femoral artery or vein.

Another remarkable difference between Löwit's and our observations is a relative temporary hyperleukocytosis which we, under normal circumstances, were never able to detect during the state of hypoleukocytosis. Löwit, however, indicates that in a restrained animal hyperleukocytosis following hypoleukocytosis within 30-60 min. is replaced by a renewed drop and the replacement of leukocytes destroyed by injection is only very incomplete. The only explanation we can give for these findings is that Löwit did not take into account cooling of the restrained animals and consequent hypoleukocytosis. Restraining alone cannot cause this incongruity, because most of our test animals were restrained often for hours, and as the numerous experiments show, neither intravenous nor subcutaneous injection produced a secondary decrease. When, after intravenous injection, the hypoleukocytosis had reached its highest point after 3, 5-10 minutes, the number of white blood corpuscles began to increase again at a constant rate. Maximum hyperleukocytosis was most often reached after 5, but frequently only after 12-18 hours; the original leukocytosis returned after 24-48 hours. Subcutaneous injections produced these phenomena more slowly; the number of white blood cells fell to a minimum only after 3-4 hours, and increased to its highest point at the earliest after 14-18 hours following the injection.

But when we disregard these few differences between Löwit's and our test /413
results, then the statement is justified that every hyperleukocytosis is preceded by hypoleukocytosis and that the former is not conceivable without the latter.

Microscopic Examination of the Organs

We approached the microscopic examinations with the intention of investigating the decay of leukocytes as stated by Löwit. We assumed that, if it really occurred upon contact of the injected material with the blood corpuscles, it should be evidenced most clearly in those organs whose capillary bed would be reached first by the substance injected into the jugular vein. Therefore, we first turned our attention to the lungs. The very first experiment gave the surprising result that a fresh lung swab in this state showed a very large quantity of leukocytes after definite hypoleukocytosis by injection of spleen extract had been produced. According to the theory of leukocytosis, very few should have been found, especially in the lungs. This caused us to examine the organs in sliced preparations. When the particular state of leukocytosis was reached at which the condition of the organs was to be examined, the thorax of the living, anesthetized animal was opened, ligatures applied to the large vessels, heart, lung, spleen, kidney, liver and bone marrow removed and prepared in the manner described above (p. 6) and examined. The heart was fixed in its entirety, of the other organs only pieces were taken. We did not inject a fixing liquid into the air passages of the lung to avoid changing the condition of the vessels. All procedures were executed as quickly as possible.

It will be useful in the description of the findings to separate them according to the various states of leukocytosis.

Examination in the State of Hypoleukocytosis After Intravenous Injection.

The capillaries of the lung (see Table II, Figure 1) are greatly filled and show besides red an unusual wealth of white blood corpuscles. In fact, the larger lumina contained less leukocytes, while very small vessels contained a very large number; in some cross sections they occasionally seemed

to be completely filled (see the small vein in Figure 1). All kinds of leukocytes were represented, predominantly polynuclear ones. The lack of white blood corpuscles in the large vessels stands in stark contrast to the leukocyte contents of the smallest vessels and capillaries. Figure 2 shows the cross /414
section of such a vessel from the same lung as in Figure 1 where leukocytes are missing entirely.

Small pieces from various parts of the lung were examined; from the hilus as well as the thin edges and the center; everywhere the same change described above could be demonstrated.

We did not find any evidence which could have been interpreted as decay.

Of course, we did not neglect to prepare control specimens from the lungs of animals which had not shown a change in the number of leukocytes. We shall discuss that later.

The obvious changes which the lung had shown could not be observed in liver, spleen, bone marrow, or kidney. It should be noted in general that the evaluation of the conditions in the bone marrow and also in the spleen are very difficult and uncertain in any case.

The capillaries of liver and kidney were filled only little or moderately and contained only a sparse amount of leukocytes. They certainly had not increased compared to the norm, much rather decreased. Also the kidney glomeruli were hardly filled, and contained few leukocytes.

Further investigation of the lungs during hypoleukocytosis, not due to spleen extract but bone marrow extract or hemialbumose, showed, as expected, but yet to be proven, that again the leukocytes were concentrated in the smallest vessels and capillaries of the lung. Also in these animals the capillaries of liver, spleen, and kidney did not show a definite increase of leukocytes.

The same results were demonstrated in an animal with hypoleukocytosis due to injection of nucleic acid.

As control, we used a rabbit with normal leukocyte count which died under chloral anesthesia even before anything else had been done to it. Another one died under ether anesthesia subject to the same conditions. With these two animals, the experimental conditions were somewhat different from those mentioned above, inasmuch as lung and heart were removed without ligating the large vessels while the heart was still beating. However, we also used a normal animal and treated it like the ones with hypoleukocytosis, namely, exenterated it under ether anesthesia.

The organs of another rabbit were removed by the method described above after it had received pancreas extract intravenously which did not produce hypoleukocytosis, as reported earlier. Also, two more animals were injected with kidney extract, also showing no hypoleukocytosis. (These last two animals died soon after the injection.)

In all these animals, the lung capillaries were filled slightly with few leukocytes, i.e., just the normal number and distribution. Compared with the preparations made from animals in a state of hyperleukocytosis, the difference left no doubt and was readily apparent. It is remarkable that the branches of the portal vein contained leucocytes more abundantly than other vessels, as observed in normal, unused animals.

Closer microscopic inspection substantiates the results of the first swab /415 — namely, that in the state of hypoleukocytosis the leukocytes are concentrated in the lung, especially in the smallest vessels and the capillaries where leukocyte destruction should have taken place primarily. Yet, in addition, there is no evidence of destruction. This result is indisputable. We wish to note especially, to anticipate the only other possible objection, that hypoleukocytosis by the method which we used is not as quickly compensated for in order to assume it had reformed during removal of the organs. Rather, the condition of the organ corresponds to maximum hypoleukocytosis. But the

result also suffices to explain the state of hypoleukocytosis in the circulating blood, because the capillary region of the lung, in addition to the smallest vessels, represents a proportion of the entire vascular system of the rabbit which is large enough to store easily a considerable percentage of the circulating leukocytes.

It must be pointed out that our test results differ from those by Michelson, whose studies given in a dissertation were not known to us at the time of our experiments. However, we believe we can trace the difference of the results to the difference of the experimental conditions. Michelson injected into the femoral vein, while we chose the jugular vein. This may have caused the fact that, in lungs which he subsequently excised, the capillaries were not as filled with leukocytes as our preparations. Nevertheless, Michelson also points out the remarkable fact that in the state of hypoleukocytosis during which the number of leukocytes in the peripheral vessels had sunk to a minimum, the lung capillaries contained a number of leukocytes which more likely exceeded the normal count.

Hypoleukocytosis Due to Cooling and Shock

We combine these two conditions here because the microscopic results for both are very similar.

In one rabbit (Experiment No. 7, p. 382) shock is produced by blows on the neck. The previous count from the ear vein amounted to 10,400 leukocytes; in the state of shock we find only 4,200. The organs are removed by the usual method. Examination of the section shows an enormous expansion and filling of the smaller and smallest blood vessels of the lungs. Also small numerous hemorrhages have occurred; the red blood corpuscles seem to be compressed in the vessels unusually tightly. A noticeable accumulation of white blood corpuscles in the capillaries is not observed. Signs of destruction cannot be shown with certainty. The vessels of the liver are filled somewhat more than usual. Heart, spleen, kidney show nothing extraordinary.

A rabbit whose rectal temperature is 38.9° C is cooled to 24.5° C by bathing in running water. The number of leukocytes in the ear vein drops from 10,400 before cooling to 3,800. The organs are taken out be the usual method; the animal had been anesthetized before cooling. The smallest vessels and capillaries of the lung are extraordinarily filled; an especially conspi- /416
cuous accumulation of leukocytes is not present. The large vessels show a noticeably low content of leukocytes. The vessels of the liver are filled somewhat more than normal. Heart, spleen, kidney, bone marrow show nothing particular.

The findings during shock and cooling, therefore, differ remarkably from other hypoleukocytoses, in that there is no noticeable packing of white blood corpuscles in the capillaries. Decay of the leukocytes probably did not take place, which is supported by the absence of products of deterioration. Under these circumstances, one could possibly conceive of another capillary area where leukocytes could accumulate which were not examined (skin ?). In this respect, experiments still have to be conducted, and this matter is therefore not concluded. Nevertheless, it is conceivable that as a result of the strong dilation of the smallest vessels and capillaries, an integral part of the white blood corpuscles is held back and occasionally taken out of circulation. This relationship may then affect the leukocytes because of their lower weight and viscosity to a higher degree than the erythrocytes.

Examination During the State of Restitution

By this, we mean the condition under which the hypoleukocytosis reverts to the initial normal number of leukocytes.

This experiment was performed on an animal after intravenous injection of hemialbumose, and resulted in revealing numerous leukocytes in the small vessels and capillaries of the lung. The preparations at once gave the impression that more leukocytes had accumulated than during hypoleukocytosis, which led to the necessary conclusion that restitution could not be merely the recirculation of leukocytes held in the capillaries.

Examination in the State of Hyperleukocytosis

The study of organs in a state of general hyperleukocytosis was made with four animals according to the same procedure. Concerning the degree of hyperleukocytosis, the number of white blood corpuscles at the time of exenteration had the following values for the individual animals:

No. 59: 27,400 leukocytes in the ear vein
No. 61: 19,000 leukocytes in the ear vein
14,800 leukocytes in the femoral vein
No. 75: 23,200 leukocytes in the femoral vein
No. 78: 24,200 leukocytes in the femoral vein

By far, the most conspicuous results were found when studying the lung. Already in the cross sections of the larger vessels there were numerous white blood corpuscles. Figure 3, Table II shows such a cross section. The contrast compared with Figure 2 is conspicuous enough. The lumina of small vessels occasionally were entirely filled with leukocytes. The capillaries were enlarged, tightly filled, and contained extremely numerous leukocytes even more than in the state of hypoleukocytosis (see Figure 4). Usually, /417
they lie in rows of 5-8. Again no signs of deterioration were observed. Only an occasional division of the indented nuclear rod into individual nuclei might be mentioned here, similar to what Ehrlich(2) saw in acute spleen tumor.

In this phase also, liver and kidney showed an increased content of leukocytes, more in the liver than in the kidney. The vessels and capillaries of the liver in general seemed fuller than usual. Anyway, the picture could not in the least be compared with that of the lung.

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(2) For the acute spleen tumor. Charite-annals. Vol. 9.

Examination in the State of Hypoleukocytosis Due to Subcutaneous Injection

Again the lung showed the characteristic finding; the increased leukocyte content of the capillaries left no doubt, even if it was not quite as considerable as after intravenous injections.

Liver and kidney showed no noticeable changes. In one animal the spinal cord was tested, and no conspicuous increase of leukocytes was found in the capillaries.

Examination After Prolonged Hyperleukocytosis

The organs of one animal were examined 48 hours after injection of spleen extract. The vessels and capillaries of the lung were very full and contained numerous leukocytes, but decidedly less than one usually sees at the peak of hypoleukocytosis. Instead, definite signs of deterioration were observed. Often in the capillaries and vessels irregularly defined neutrophilic protoplasmic masses with faintly stained indications of nucleus fragments, also separate parts of nuclei.

The capillaries of the liver were quite filled; the proportion of leukocytes seemed neither more nor less. No signs of deterioration. The same was true of the kidney. In the spleen numerous leukocytes; no distinct signs of deterioration.

Another animal, having received 4 cc of spleen ferment, showed 22,400 leukocytes in the ear vein 20 hours after the injection; after 66 hours — 48,800 leukocytes. In this condition, the organs are taken out under anesthesia. In the lung sections, numerous leukocytes, but not as crowded as in fresh hyperleukocytosis. Here again the conspicuous frequency of broken nuclear rods and separately situated nucleus fragments were observed. These may certainly be considered as signs of the deterioration process.

The liver showed a notable result, because the quantity of leukocytes was considerably increased in the larger and smaller vessels as well as in the capillaries. Also in other cases, the increase of white blood corpuscles in the liver due to hyperleukocytosis had been observed (see above), but in this case, the relationship to the lung seemed changed. The number of leukocytes in the liver seemed larger than in fresh hyperleukocytosis, smaller in the lung. This is probably the explanation: the number of leukocytes found in the liver in this case is just about equal to the high degree of hyperleukocytosis which existed in this animal. But in fresh hyperleukocytosis /418 the lung contains, in addition, the deposit of retained white blood corpuscles which now — after prolonged duration of the hyperleukocytosis — is reduced (due to deterioration). Spleen and kidney show nothing special.

Finally, a microscopic examination was performed on an animal which had died five days after a subcutaneous injection of 8 cc nucleic acid. Three days after the injection, 18,400 leukocytes were counted. The lung contained numerous leukocytes, just about to the degree to which one would find them during hypoleukocytosis, i.e., fewer than during fresh hyperleukocytosis. We noticed that the nuclei of the leukocytes of all preparations did not stain as deeply with triacid as was usually the case, and that their contours were not as sharply defined as in the normal case. However, it is questionable if this circumstance should be considered significant, since the organs were not fixed while still alive, but were taken from the dead animal. We also found deteriorated nucleus masses. The erythrocytes were well-stained. The change of the white blood corpuscles in the capillaries requires further examination, even if our findings permit us to express the opinion that a part of them anyway deteriorates.

The smallest vessels and capillaries of the liver showed a minor increase of leukocytes; spleen and kidney — nothing special.

Examination in the State of Hypoleukocytosis Produced After Previous Strong Hyperleukocytosis by Injection of Bacteria

As already mentioned, a massive intravenous injection of bacteria even during the state of strong hyperleukocytosis produces an enormous decrease of leukocytes in the blood, sometimes even reaching a near absence of white blood corpuscles in the circulating blood. For large number of such injection experiments, the organs had been taken out and examined. For these, the lungs showed almost an overflow of leukocytes (see Figure 5). In the capillaries which form a tightly knit mesh, the red blood corpuscles are almost replaced by leukocytes which were stacked in long rows. Occasionally, the cut had the appearance of a purulent inflitration because of the dense masses of polynuclear cells. The smallest vessels often seemed completely clogged with white blood corpuscles, while the large vessels were almost completely devoid of them. Several times a clot of cohering leukocytes was found attached to the wall of a larger vein. Here and there, the endothelium — that is, the vessel wall — had the smallest tears, since the lung tissue seemed in places to be infiltrated throughout by red and white blood corpuscles. Once a mass of leukocytes mixed with red blood corpuscles and several endothelial cells were found in a bronchiolus.

Also, in the liver the leukocytes in the capillaries and the smallest vessels were increased; in the animal injected with the potato bacillus and putrefaction bacillus, there was a considerably increased leukocyte content in the liver, so that often 3-4 corpuscles were lined up. Nevertheless, there was no comparison with the appearance of the lung. Once a large mass of leukocytes was observed in the cross section of a small portal artery. Signs of deterioration were not demonstrable either in the lungs or the liver. The kidney never showed any increase in leukocytes.

Microscopic examinations were also performed on animals with a combination of treatments. Thus, we used animal No. 82 which had first received subcutaneous spleen extract, and after hyperleukocytosis set in — intravenous

spleen extract. The number of leukocytes had dropped after the second injection, and then had risen to just about normal when the animal died. The lung showed numerous leukocytes. /419

Similarly, animal No. 80 had hyperleukocytosis produced by subcutaneous injection, then hypoleukocytosis by intravenous injection, which finally had changed into moderate hyperleukocytosis.

Animal No. 74 had received intravenous spleen extract injection which was repeated during the period of increasing leukocytosis. Soon thereafter, death occurred while the number of leukocytes had almost reached normal again. The lungs showed a large content of leukocytes.

Animal No. 71 received intravenous injection during the state of hypoleukocytosis produced by intravenous injection of spleen extract. When the animal was killed, the number of leukocytes had not yet reached normal. Few leukocytes were found in liver and kidney, while the lungs presented the usual picture of leukocyte accumulation.

One animal, after previous subcutaneous injection of spleen extract, had received an intravenous injection during the initial phase of increasing leukocytosis which finally produced a strong hyperleukocytosis. The lungs showed a tremendous wealth of leukocytes.

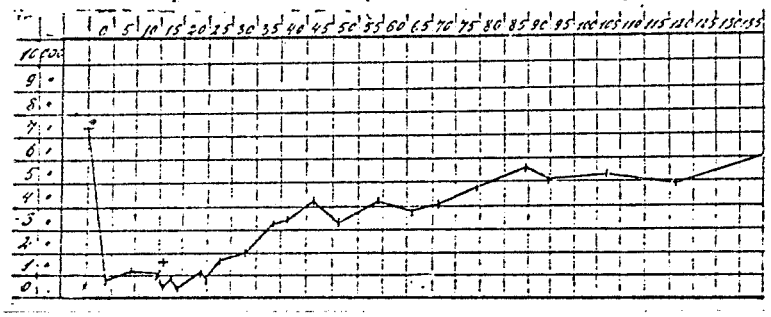
These findings show that even after combined treatment, the microscopic conditions always corresponded to those achieved after single injections.

Combination of Different Injections

After we had reached the results just reported by microscopic examination, we had to approach the question of chemotactic influences. For this purpose, we first made observations to determine if any and what signs would be produced when, after one injection to change the leukocytosis, the injection is repeated. Furthermore, we examined the effect of excessively high doses

upon leukocytosis. Then we examined the way in which a substance for injection affected the number of leukocytes after the same substance had been introduced into the pulp. Finally, we injected small doses of the same substance within small time intervals.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
70	Intravenous injection in the state of hypoleukocytosis	Injection of 8 cc spleen extract into left ext. jug. vein	October 3 1:29.30 pm 1:29.45 pm 1:32.55 pm 1:38.5 pm	7200 Inj. 6800 800 1200	
					20 hours after the injection, 25400 leukocytes in the ear vein.



At • intravenous injection of 8 cc spleen extract;
at + another intravenous injection of 8 cc spleen extract.

	8 ccm injection into the same vein	1:43.40 pm	Inj.
		1:44.— pm	1200
		1:46.10 pm	400
		1:48.5 pm	800
		1:49.55 pm	200!
		1:52.18 pm	1200
		1:53.— pm	1000
		1:56.55 pm	1600
		1:58.15 pm	2200
		2: 2.45 pm	2000
		2: 5.— pm	3400

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count*	General remarks
			2:8.15 pm	4000	
			2:11.5 pm	3600	
			2:15.15 pm	4200	
			2:17.40 pm	4000	
			2:20.45 pm	3400	
			2:28.40 pm	4200	
			2:35.35 pm	3800	
			2:39.15 pm	4000	
			2:44. 5 pm	4600	
			2:54.10 pm	5400	
			2:58.50 pm	4800	
			3:10.15 pm	5200	Even after two hours and nine minutes, not the normal number of leukocytes.
			3:22.— pm	5000	
			3:38.50 pm	6400	
			3:47.10 pm	Ear vein 9700	
			October 4		
			10:—.— pm	Ear vein 25400	Animal well.
71			December 8	5800	
	As No. 70	Injection of 8 cc spleen extract into left external inuinal vein	2:31.— pm	Inj.	
			2:36.30 pm	1200	
			2:37.— pm	1400	
		Injection of 8 cc spleen extract into same vein.	2:39.30 pm	Inj.	
			2:41. 5 pm	1000	
			2:43.30 pm	600	
			2:45.10 pm	1200	
			2:48.50 pm	1400	
			2:53.30 pm	1200	
			2:58.— pm	2000	
			3: 2.55 pm	2400	After 1 hour 17 min. still strong hypo-leukocytosis.
			3:10.45 pm	3200	
			3:20.20 pm	2400	

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*The number of leukocytes refers to the count made from the femoral vein, unless otherwise noted.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			3:34.20 pm	3000	
			3:48.45 pm	3400	Animal is killed for microscopic examination.
			November 13		
72	As No. 70		1:30.— pm	Ear vein 10200	
		Injection of 6 cc spleen extract subcutaneously into abdominal skin	1:45.— pm	Inj.	
			5:30.— pm	ditto 3800	
			5:58.— pm	ditto 3000	
		Injection 7 1/2 cm spleen extract into left external jug. vein.	6: 3. 5 pm	Inj.	
			6: 4.43 pm	1200	
			6: 6.12 pm	1400	
			6:11.41 pm	800!	
			6:15.38 pm	—	Even after 53 min., strong hypoleukocytosis.
			6:19.18 pm	1200	
			6:29.25 pm	1800	
			6:31.36 pm	2400	
			6:41.52 pm	2000	
			6:49.28 pm	2400	
			6:56.56 pm	2800	The animal is sacrificed for microscopic examination.
78	Intravenous injection when increasing leukocytosis is just beginning		2:30.— pm	6200	
		Injection of 8 cc spleen extract into left ext. jug. vein.	2:42.10 pm	Inj.	
			2:45.40 pm	2200	
			2:57.50 pm	2000	
			3: 6.— pm	3000	
		Injection of 8 cc spleen extract into the same vein.	3:13.30 pm	Inj.	After the second injection, not only do the leukocytes fail to reduce, but a quick increase sets in, reaching 3 1/2 times the original number in 46 1/2 min.
			3:15.20 pm	6600	
			3:18.40 pm	8000	
			3:25.10 pm	10600	
			3:35.— pm	16800	
			3:45.10 pm	18200	
			3:50.— pm	20800	
			4:—.— pm	22000	
			4:18.30 pm	24400	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr · min sec	Leukocyte count	General remarks
74	Same as No. 73		2:—.— pm	7800	
		Injection	2:12. 8 pm	Inj.	
		of 8 cc	2:15.30 pm	2200	
		spleen ex-	2:17.40 pm	1800	
		tract into	2:20.35 pm	2200	
		left ext.	2:24. 5 pm	3000	While after the /422
		jug. vein.	2:27.40 pm	3400	first injection a
			2:34. 5 pm	3600	clear hypoleuko-
			2:38. 5 pm	5000	cytosis appeared
		Injection	2:42.24 pm	Inj.	within three
		of 8 cc	2:43.10 pm	6000	minutes. the
		spleen ex-	2:44. 5 pm	5600	second inj. pro-
		tract into	2:45.— pm *	—	duced no notice-
		left ext.	2:46.10 pm	6800	able decrease.
		jug. vein.			*Breathing ceases.
			2:47.— pm	death	Animal dies spontaneously.
75			December 5		
	Same as		5:45.— pm	6000	
	No. 73	Injection	5:52.15 pm	Inj.	
		of 8 cc	5:55.45 pm	2000	
		spleen ex-	5:58.17 pm	800!	
		tract into	6: 3.55 pm	1600	
		left ext.	6:10. 5 pm	2600	
		jug. vein.	6:14.50 pm	3600	
		ditto	6:18.25 pm	Inj.	
			6:19.43 pm	4200	After the second
			6:20.35 pm	3800	injection, instead
			6:22.30 pm	4400	of a decrease, leuko-
			6:22.30 pm	6400	cytosis increased
			6:24.35 pm	8600	sharply, amounting
			6:29.— pm	10800	to four times the
			6:31.45 pm	12400	original number after
			6:37.53 pm	18400	53 minutes.
			6:48.30 pm	20400	
			7:11.15 pm	23200	Animal is killed for microscopic examination.
			October 24	Ear vein 9800	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
76	Same as No. 73.	Injection of 10 cc spleen extract subcutaneously into abdominal skin.	8:—.— am 1:43.— pm	Inj. 4400	
		Injection of 10 cc spleen extract into the left ext. jug. vein.	1:57.— pm 1:57.45 pm 2.—.25 pm 2: 3.55 pm 2: 7.30 pm 2:11. 5 pm 2:15.30 pm 2:22.30 pm 2:27.50 pm 2:35.45 pm 2:47.30 pm 2:50.25 pm 2:54.15 pm 2:58.10 pm 3: 3.10 pm 3: 8.30 pm 3:14. 5 pm 3:20.35 pm 3:29.30 pm 3:39.30 pm 3:51.45 pm 3:57. 5 pm 4: 4.10 pm 4:18.10 pm 4:20.35 pm 4:34.30 pm 4:47.30 pm 4:57.30 pm 5:11.15 pm 8:—.— pm	Inj. 4000 5600 7400 9800 10400 11000 13600 15200 14600 8800 7000 7800 8200 7400 7200 4200 5200 4600 4200 2600 3000 3800 5200 5800 7000 17600 10200 14800 Ear vein 25400 Ear vein 10400	—

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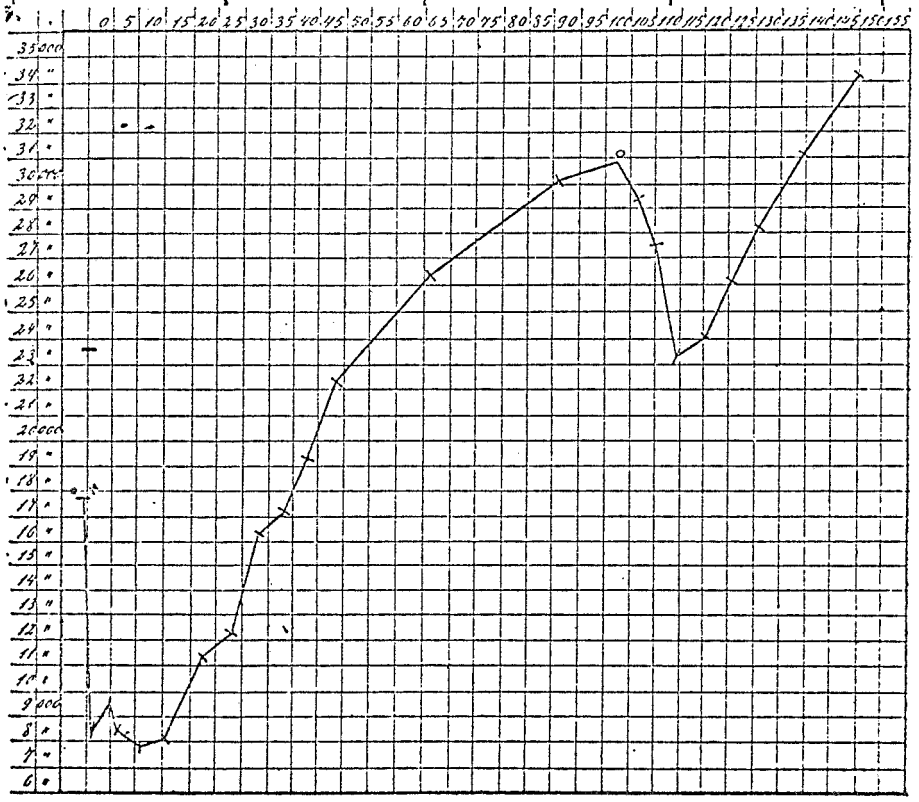
Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			October 5	5,440000 Eryth.	
77	Intravenous during already existing hyperleukocytosis	Injection of 8 cc spleen extract into left ext. jug. vein ditto	10:—.— am 11:30.— am 2:45.— pm 2:47.— pm 2:48.30 pm	Inj. Ear vein 2800 Ear vein 23600 17800 Inj. 8400	



4 3/4 hours before start of curve, intravenous injection of 8 cc spleen extract. At +, second intravenous injection of spleen extract. At °, the same a third time.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			2:52.20 pm	9600	/424
			2:55.45 pm	8600	
			2:58.30 pm	7800!	Maximum decrease
			3: 2.— pm	8200	of leukocytes after
			3: 6.20 pm	11600	second injection is
			3: 9.45 pm	12800	reached after 11
			3:13.30 pm	16400	min. Already after
			3:18.— pm	17400	40 min. 55 sec., the
			3:21.15 pm	19200	degree of hyper-
			3:25.30 pm	5,800000 eryth.	leukocytosis exist- at the time of the
			3:27.55 pm	22600	second injection has
			3:45.30 pm	26400	again been reached.
			3:55.30 pm	Ear vein 38400	
			4:15.30 pm	30400	
		Injection	4:32.— pm	Inj.	
		of 8 cc	4:35.15 pm	29600	After the third
		spleen ex-	4:38.— pm	26200	injection only low-
		tract into	4:40.50 pm	23200!	grade reduction of
		left ext.	4:45.10 pm	24200	number of leukocytes.
		jug. vein.	4:52. 5 pm	26400	8 min. 50 sec. later,
			4:58.15 pm	28200	after 40 min. 30 sec.,
			5:12.30 pm	31200	the number is back to where it was at the time of the third injection.
			5:15.— pm	34400	The experiment shows exhaustion of the
			October 6		depressive action
			10:—.— am	Ear vein 24600	(action on hypo- leukocytosis) of repeated injections during existing hyperleukocytosis.
78	Same as No. 77.		9:—.— am	Ear vein 8800	Incision is sewn up, animal quite well.
		Injection of 8 cc spleen ex- tract into left ext. jug. vein		Inj.	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			12:45.— pm	Ear vein 19800	
		ditto	1:16.40 pm	Inj.	Animal reanesthetized and operated.
			1:17.18 pm	12000	
			1:18.50 pm	9600	Maximum of low-grade decrease of leukocytes following second injection is reached after 5 minutes. Already after ca. 30 min. is the degree of hyperleukocytosis reached which existed at the time of the second injection.
			1:21.45 pm	8800	
			1:24. 5 pm	10000	
			1:26.50 pm	12400	
			1:30. 5 pm	15600	
			1:36.— pm	19200	
			1:39.40 pm	20800	
			1:44.35 pm	22400	
			1:54.— pm	23600	
			2:—.— pm	24200	The animal is killed for microscopic examination.
			October 13		
79	Same as No. 77.	Injection of 10 cc spleen extract subcutaneously into abdominal skin.	7:—.— pm	Inj.	/425
			October 14		
			1:—.— pm	Ear vein 27400	
		Injection of 8 cc spleen extract into left ext. jug. vein.	1:59.— pm	Inj.	
			2:1. 24 pm	4800	After strong hyperleukocytosis is achieved by subcutaneous injection, another intravenous injection greatly decreases number of leukocytes.
			2: 3.30 pm	3200	
			2: 6.— pm	2200	
			2: 8.25 pm	2800	
			2:11.10 pm	1800!	
			2:14.10 pm	3000	
			2:17.15 pm	3800	
			2:21.15 pm	5400	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			2:26.10 pm	5800	Only after 1 hr.
			2:38. 5 pm	10600	10 min., the degree
			2:46.15 pm	15800	of hyperleukocytosis
			2:56.40 pm	16600	existing at the time
			3: 8.50 pm	20800	of the second injec-
			3:30.25 pm	31200	tion has been reached.
		Injection of 1 cc very concentrated aqueous suspension of putrefying bacteria.	4:——.— pm	Inj.	An injection of putrefaction bacteria produces an enormous hypoleukocytosis.
			4:20.24 pm	1800	The animal is killed
			4:31.— pm	800!	for microscopic examination.
			October 28		
80	Same as No. 77.	Injection of 8 cc spleen extract subcutaneously into abdominal skin.	8:—.—	Inj.	
			October 29		
			5:15.— pm	Ear vein 20800	
		Injection of 8 cc spleen extract into left ext. jug. vein.	5:21.25 pm	Inj.	
			5:22.35 pm	7600	After hyperleukocytosis
			5:21.30 pm	5800!	is achieved by subcu-
			5:27.— pm	6600	taneous injection, an
			5:30.55 pm	7800	intravenous injection
			5:33.35 pm	10800	leads to strong and
			5:37.30 pm	14800	rapid decrease. This
			5:43.35 pm	14000	is soon followed by
			5:48.15 pm	15600!	increase of leukocytes
			5:54.45 pm	10600	up to original amount
			5:59.40 pm	11400	of hyperleukocytosis,
			6: 3.50 pm	9600	but then without further
			6:10.25 pm	9200	treatment a renewed drop
			6:15.30 pm	10400	and finally again an increase.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			6:22.37 pm	9800	
			6:30.15 pm	12000	
			6:35.55 pm	13600	The animal dies.
			November 17		/426
81	Same as No. 77.	Injection of 10 cc spleen extract subcutaneously into abdominal skin.	1:—.— pm	Inj.	
			November 18		
			1:30.— pm	22400	
		Injection of 8 cc spleen extract into left ext. jug. vein.	1:49.— pm	Inj.	
			1:49.30 pm	15200	
			1:51.10 pm	7800	
			1:53.25 pm	5800	
			1:56.50 pm	5200!	
			2: 1.— pm	6600	
			2:10.— pm	9800	
			2:21.9 pm	12000	
			2:33.10 pm	17200!	Under the same conditions, the same peculiar sequence of leukocytosis takes place; only the secondary decrease is still steeper.
			2:54.— pm	8200	
			3: 5.15 pm	2200!	
			3:12. 2 pm	2800	
			3:17.20 pm	5400	
			3:23.10 pm	4800	
			3:31.29 pm	5800	
			3:44.30 pm	6200	
			3:54.15 pm	8000	
			4: 7.— pm	9400	
			4:26.— pm	10200	
			4:40.— pm	9800	
			November 19		
			10:30.— am	Ear vein 23600	
			October 20		
82	Same as No. 77.	Injection of 8 cc spleen extract subcutaneously into abdominal skin.	5:—.— pm	Ear vein 10600 Inj.	

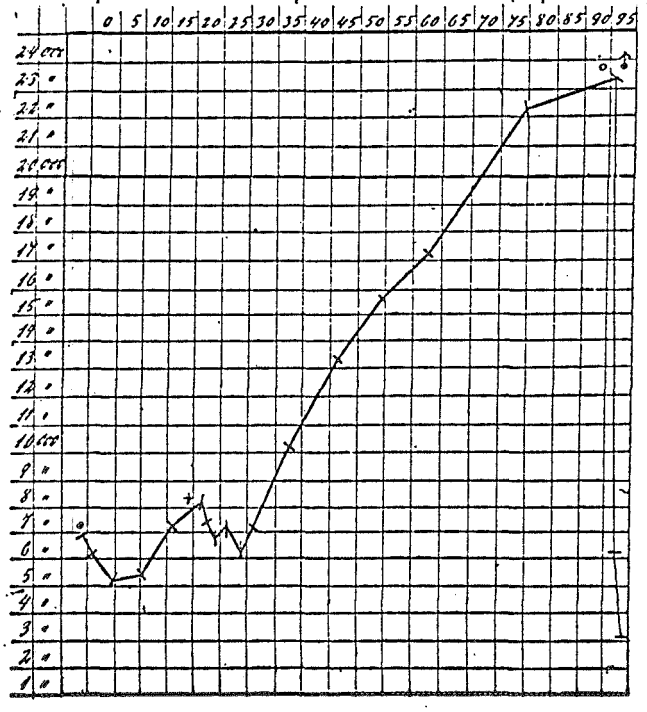
Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			October 21	ditto	The rabbit board was
				22600	carelessly overheated,
			2:30.— pm	16800	temperature of the
		Injection	2:54.30 pm	Inj.	animal was 43° C;
		of 8 cc	2:56.10 pm	9400	experimental animal
		spleen ex-			suffered from very
		tract into			acute heat dyspnea
		left ext.			and died from it.
		jug. vein			
			2:59.55 pm	3000	Strong decrease
			3: 6.— pm	2400	after second
			3:13.30 pm	4600	injection.
			3:23.50 pm	6800	Animal dies.
			October 10	10600	
83	Same injec-	Injection	1:38.— pm	Inj.	
	tion of an	of 20 cc	1:39.— pm	8400	
	excessively	spleen ex-	1:42.40 pm	2400!	Maximum hypoleuko-
	high dose.	tract into	1:45.35 pm	2800	cytosis 4 min 40 sec
		ext. jug.	1:49.15 pm	9800	after injection;
		vein	1:52.15 pm	12200	already 14 min later
			1:54.45 pm	15800	more than the origi-
			1:57.55 pm	16400	nal number; rapid
			2: 2.50 pm	17800	increase
			2: 7.25 pm	Ear vein	/427
				22800	
			2:16.40 pm	18800	
			2:25.35 pm	17400	
			2:34.45 pm	17800	
			2:47.30 pm	18200	
			3: 5.50 pm	19200	
			October 11		
			1:30 pm	Ear vein	
				3800	Animal is sewn up,
					is well.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
		Curve for No. 83			
					At*, intravenous injection of 20 cc spleen extract.
				6200	During the injection, a clamp above the site of injection on the jug. vein comes loose causing considerable loss of blood which seemingly affected the rapid appearance of hyperleukocytosis.
84	Same as No. 83.	Injection of 20 cc spleen extract into ext. jug. vein.	2: 9.— pm	Inj.	
			2:11.— pm	2600	
			2:13.15 pm	1800!	
			2:15.40 pm	2000	
			2:18.15 pm	3200	
			2:21.30 pm	3800	
			2:24.50 pm	4200	
			2:31. 5 pm	4400	
			2:43.50 pm	5400	
			2:55.15 pm	5800	
		Injection of ca. 3 cc into same vein (some of it into surrounding tissues).	3: 4.30 pm	Inj.	
			3: 5. 5 pm	4200	
			3: 7.35 pm	5200	
			3:10.50 pm	5600	
			3:16.— pm	6000	
			3:21.30 pm	7200	
			3:27.30 pm	8200	
			3:35.30 pm	9600	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
					/428
		Injection of 6 cc very concentrated suspension of pneumococci.	3:51.30 pm 3:51.50 pm 3:53.15 pm	Inj. 10800 1600!	Animal is killed for microscopic examination.
			October 18	7000	
85	Same as No. 83.	Injection of 20 cc spleen extract into ext. jug. vein.	1:59.45 pm 2:—.20 pm 2: 1.50 pm 2: 5.45 pm 2: 9.— pm 2:11.30 pm 2:15.55 pm 2:29.30 pm 2:30.20 pm 2:44.25 pm 3: 1.50 pm 3:22.37 pm 3:45.44 pm	Inj. 1200 1200 1600 1000! 2400 3800 5200 4800 5800 6400 7200 8800	Excision of a large piece of a femoral vein for fixing. At that time, in spite of great care, rather considerable blood loss which probably caused the slow increase of leukocytes. Animal dies.
86	Same as No. 83.	Injection of 20 cc spleen extract into ext. jug. vein.	2:22.— pm 2:24.25 pm	7400 Inj. 2200!	Immediately after injection the animal becomes extremely dyspnoic and dies shortly thereafter since the injection was executed too rapidly.
				Ear vein 9800	
87	Injection of a larger dosis following a smaller one.	Injection of 1 cc spleen extract into ext. jug. vein.	October 30 2:19.30 pm 2:21.30 pm 2:25.10 pm 2:30. 5 pm 2:34.55 pm	6800 Inj. 6400 5200! 5400 7400	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
				Inj.	
		Injection of 8 cc of same substance into same vein	2:41.— pm	8400	After achieving a slightly decreasing and then increasing leukocytosis after injection of 1 cc, a second larger injection given directly should produce a strong decrease of leukocytes, but results in only low-grade decrease (-8 min later); then rather rapid increase (after ca. 40 min more than triple). Animal is killed for microscopic examination.
			2:41.30 pm	7800	
			2:42.55 pm	6800	
			2:44. 5 pm	7200	
			2:45.50 pm	6200!	
			2:49.— pm	7000	
			2:50.30 pm	10200	
			2:54.10 pm	13400	
			2:57.30 pm	15800	
			3: 4.30 pm	17200	
			3: 9.35 pm	22400!	
			3:20.35 pm	23200	
		Injection of very dense suspension of potato bacteria.	3:35.45 pm	Inj.	
			3:45.— pm	6200	
			3:46.— pm	3200!	

Curve for No. 87

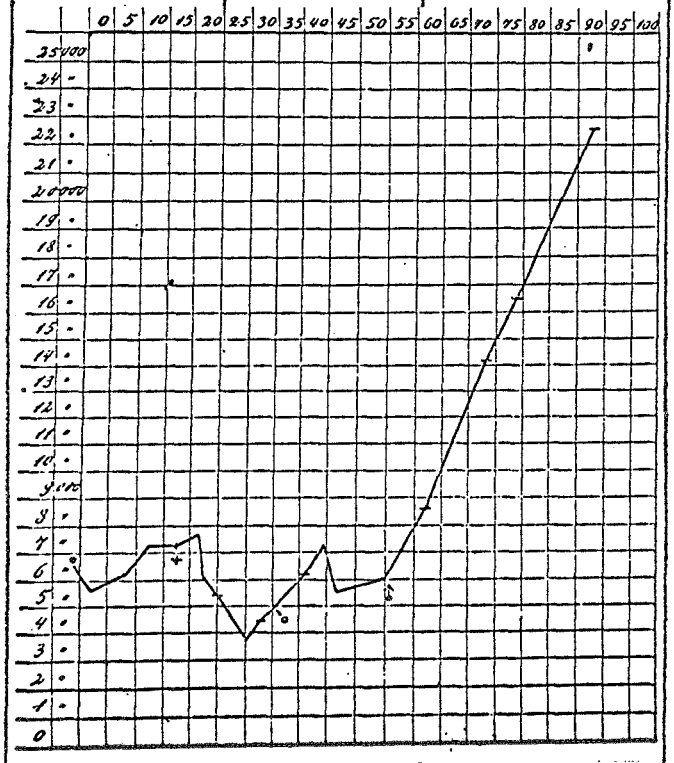


/429

At °, intravenous injection of 1 cc spleen extract.
At +, intravenous injection of 8 cc spleen extract.

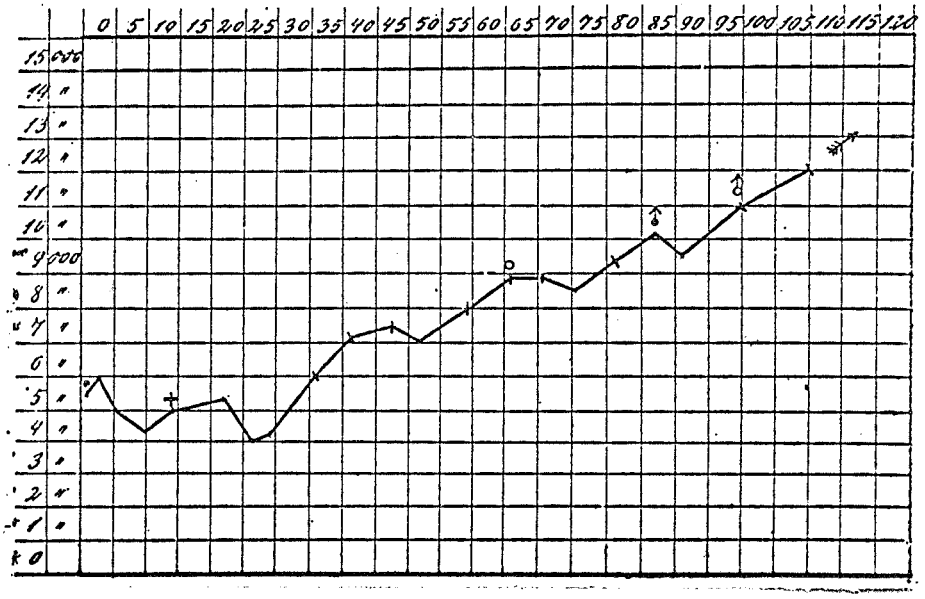
Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
88	Same as No. 87		November 2	6800	
		Injection of 1 cc spleen extract into jug. vein.	2:28.10 pm	Inj.	
			2:30.15 pm	7400	
			2:34.15 pm	6200	
			2:38.20 pm	5800	
			2:43.— pm	5000!	
			2:47.10] pm	5800	
		Injection of 8 cc spleen extract into ext. jug. vein.	2:55.45 pm	Inj.	
			2:56.40 pm	6400	
			2:57.50 pm	4200	
			3:—.20 pm	4400	
			3: 1.45 pm	3800!	
			3: 3.10 pm	5800	
			3: 5.20 pm	7200	
			3: 9.35 pm	8000	
			3:12.20 pm	8600	Test results same as in No. 87.
			3:20.50 pm	10200	
			3:27.40 pm	12400	
			3:34.— pm	13800	
			3:51.— pm	15400	
			4: 5.— pm	17600	
			November 8		
			10:10.— am	Ear vein 20800	Animal is sewn up, and is well.
			November 3	6600	/430
89	Injection of small doses in small time intervals.	Injection of 1 cc spleen extract into ext. jug. vein	3:42.27 pm	Inj.	
			3:43.54 pm	3800!	
			3:49.15 pm	6200	
			3:54.39 pm	7200	
			4:—.34 pm	7400	
		again 2 cc	4: 6.45 pm	Inj.	
			4: 7.— pm	7800	
			4: 8.47 pm	6200	
			4: 9.50 pm	5400!	The small doses effect only a lowgrade decrease of leukocytes. Finally, an unproportionately high hyperleukocytosis
			4:14.53 pm	3800	
			4:17.42 pm	4600	
		again 2 cc	4:22.50 pm	Inj.	
			4:24.10 pm	6200	
			4:26.22 pm	7200	

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			4:28.53 pm	5400!	sets in.
			4:34.57 pm	6000	
		again 1/2 cc	4:40.23 pm	Inj.	
			4:41.22 pm	8600	
			4:46.33 pm	14400	
			4:50.27 pm	16800	
			5: 1.51 pm	22600!	
			November 4	Ear vein 28200	
			9.—.— am		
			6:20.— pm		The animal is sewn up and is well.



At °, intravenous injection of 1 cc spleen extract.
At +, intravenous injection of 2 cc spleen extract.
At °, intravenous injection of 2 cc spleen extract.
At ↕, intravenous injection of 1/2 cc spleen extract.

Exp. No.	Description of experiment	Manner of treatment	Time of day hr min sec	Leukocyte count	General remarks
			November 6	5400	/431
90	Same as No. 89.	Injection of 1 cc spleen extract into ext. jug. vein.	3:43.10 pm	Inj.	
			3:44.15 pm	6000	
			3:48.10 pm	5000	
			3:52.50 pm	4400!	
			3:57.50 pm	4800	
			4: 2.— pm	Inj.	
		the same	4: 3.20 pm	5400	
			4: 7.30] pm	3800!	
			4:10.30 pm	4200	
			4:14.50 pm	6200	
			4:20.20 pm	6200	
			4:24.10 pm	7800	
			4:28.50 pm	7600	
			4:34.30 pm	8400	
			4:42.30 pm	8800	
		the same 1/4 cc	4:47.40 pm	Inj.	
			4:48.50 pm	9000!	Test results same as in No. 89.
			4:53.20 pm	8600!	
			4:59.— pm	9400	
		the same 1/2 cc	5: 2.30 pm	Inj.	
			5: 4.50 pm	10000	
			5: 8.48 pm	9600	
		the same 0.1 cc	5:14.40 pm	Inj.	
			5:16.10 pm	10800	
			5:20. 5 pm	11400	
			5:29.35 pm	11800	
			November 7		
			9:45.— am	Ear vein 19800	Animal is sewn up and is well.



At ˙ , intravenous injection of 1 cc spleen extract. At + , likewise. At ° , intravenous injection of 1/4 cc spleen extract. At ↑ , intravenous injection of 1/2 cc spleen extract. At ↑ , intravenous injection of 0.1 cc spleen extract.

Summarizing the results of the preceding experiments, we have found the /432 following:

When the same substance is injected in about equal doses at a time when — after the first intravenous injection — hypoleukocytosis is strongly developed, it is considerably lengthened in its duration. The increase takes place only gradually, much more slowly than after a single injection. The relationship is analogous when the first injection is given subcutaneously, and the second is given intravenously at the time of hypoleukocytosis produced by the first injection.

Changing experimental conditions to giving the second injection after reduction of leukocytes (due to the first injection) has passed its peak and is ascending, a completely different situation is encountered. A second hypoleukocytosis can hardly be observed; leukocytosis climbs constantly and steeply. However, if the first injection is given subcutaneously, then the second also produces a rapid increase, provided it is applied according to the aforementioned conditions. However, it soon gives way to a secondary decline, which lasts a short while, until finally considerable hyperleukocytosis sets in.

Finally, when the second injection is given during the state of fully developed hyperleukocytosis, another hypoleukocytosis is produced, but relatively far less and of considerably shorter duration than the same doses as primary injection. Even when the first injection is given subcutaneously, the chronological development is similar, except that the hypoleukocytosis is more considerable than in the first case and that also here occasionally a secondary decrease appears. But when the second or rather the third injection instead of the same substance contains another one — e.g., suspension of putrefaction bacteria, as was done in other investigations which need not be discussed further[3], very soon it produces hypoleukocytosis down to a few

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[3] Compare R. F. Müller, on the reaction of leukocytes to injections of bacteria. Inaug. Diss., Berlin, 1894.

thousand, even less than a thousand, in spite of extremely high hyperleukocytosis.

In another series of experiments, we injected the entire amount of the substance previously distributed over various time periods, ca. 20 cc, i.e., 2 to 3 times the usual dose intravenously all at once. The result of this /433 injection is a considerably shorter duration of hypoleukocytosis than usual. Restitution sets in much more rapidly, and subsequent hyperleukocytosis increases extremely steeply.

If the particular substance is put into the liquid mass in smaller amounts and the same substance in the usual dose is injected after some time, a remarkable difference is seen in the chronological sequence of events: After the second injection, only a minor, quickly passing hypoleukocytosis occurs. The hyperleukocytosis, however, is all the more pronounced.

Finally, we examined what happens when small doses of the same substance are repeatedly injected within short time intervals. The result showed that under these experimental conditions, it is possible to avoid the state of hypoleukocytosis almost completely, whereas after the third to fourth injection, intensive hyperleukocytosis gradually sets in.

These are the results of the experiments mentioned in the last chapter. In the following, we will now attempt to deduce from these a concept of the nature underlying the changes of leukocytosis.

The Nature of Changes in Leukocytosis

First, we wish to present our views on the nature of changes in leukocytosis in order to base them on our experiments. The views which we were led to adopt on the basis of our experimental results are as follows:

"Hypoleukocytosis depends on a process which consists of leukocytes — preferably polynuclear ones — being driven into the capillaries and the

smallest blood vessels of certain organs and held there. Destruction of white blood corpuscles during this phenomenon is only of subordinate significance, if any at all. Hyperleukocytosis is produced by a substance entering the lymphatic system causing an increased transport of white blood corpuscles into the blood stream. Formation of leukocytic elements is probably involved only to a slight degree. Hyperleukocytosis seems to be primarily the result of numerous elements from blood-producing organs — especially the bone marrow, including enough polynuclear ones which are stored there for ready release — /434 entering into the blood stream as a consequence of the particular treatment. The two processes, causing hypo- and hyperleukocytosis, are therefore in themselves independent of one another. They are also not mutually exclusive; rather, the first still continues, while the second is already formed, producing a leukocyte count which is actually the result of the interplay of two opposing processes. The same phenomenon will therefore arise in conformity with one or the other, depending on the particular conditions.

This explanation, however, does not include the phenomena produced by cooling or shock.

As far as these are concerned, we believe the resulting hypoleukocytosis is caused by the tremendous filling of the smallest vessels and capillaries, where a part of the leukocytes is gradually retained. For the rest, we refer to p. 415 ff.*

The relatively lowgrade hypoleukocytosis later on, which follows a long time after cooling is terminated, can perhaps be explained by the local conditions of irritation as a consequence of leukocyte-accumulation in the capillaries, as Lassar [25] has mentioned in his experimental work on colds. This may cause an increased export of leukocytes from the blood-forming organs.

Now let us examine what other authors say about the nature of leukocytosis. Returning to Schulz' theory, it becomes apparent from the results presented in the last chapter and the reasons already mentioned, how untenable this theory is. Schulz states that hypoleukocytosis is not the consequence of

*Translator's note: This is English page 51.

leukolysis. This assumption, according to our investigations, is indeed correct. The explanation, however, which Schulz gives for the appearance of hypoleukocytosis is erroneous. He believes that the minority of leukocytes found in the peripheral vessels is compensated by a corresponding increase in the central vessels. This is inadmissible. In a part of the capillary system leukocytes are accumulated in masses. Conversely, Schulz explains hyperleukocytosis by assuming hypoleukocytosis in the central vessels, when the peripheral ones are observed to contain an increase — to say nothing of an absolute increase of white blood corpuscles. We have already shown that this statement is refuted by the results of the experiments. Once more, we point out that such an absolute increase indubitably takes place, and hypo- /435
leukocytosis cannot be explained by the liberation of the leukocytes that are held in the capillaries during hypoleukocytosis. As our preparations clearly indicate, we found a much stronger accumulation of white blood corpuscles in the capillaries during the state of hyperleukocytosis, literally the formation of thrombi which consisted first of leukocytes held earlier, and second — of those newly introduced.

Concerning Limbeck's theory, it cannot be considered valid, because v. Limbeck did not take hypoleukocytosis into consideration. The reason for this may be that his first count is always 4-6 hours after injection. Furthermore, we cannot acknowledge the connection between exudation and leukocytosis. Admittedly, hyperleukocytosis is a by-product of the former; however, it occurs without exudation. Our experiments show that subcutaneous injections of organ extracts never produced a swelling, nor formation of exudate, and they still produced a considerable hyperleukocytosis. Limbeck's theory might be useful in one respect — namely, that after hypoleukocytosis due to cooling, hyperleukocytosis follows. If the accumulation of white blood corpuscles in the capillaries is considered a local inflammation process and later on they cause an increased export or leukocytes from the blood-producing organs, in the sense of v. Limbeck's theory one can assume that inflammation and hyperleukocytosis are connected. But in no way can one state that the former by itself causes the latter, as is sufficiently evidenced by our examinations of the specific effective substance.

Concerning Römer's theory, an essential difference exists between his and our assumptions concerning the origin of leukocytes during hyperleukocytosis. Römer believes that the increase is caused by a direct generation of white blood corpuscles touched off by the injected substance — namely, exclusively in venous blood. We shall not discuss the reasons in detail with which Römer supports his theory, since we agree with Löwit's counter evidence mentioned in the particular chapter in his book which we refer to, and in connection with our examinations. We only wish to emphasize that we consider Römer's experiment with the amputated ear, following Rieder's and Löwit's similar experiments, as completely irrelevant, and we must state that the /436 given difference in the number of leukocytes in the arterial and venous area does not exist. Obviously, depending on the experimental conditions, the choice of the injection site and the direction of the injection with regard to the heart temporary variations in the number of white blood corpuscles in the arteries and corresponding veins will appear (see experiment 57). These differences, however, are soon evened out. We find hyperleukocytosis in the arteries, as well as in the veins, even if only to a slightly lower degree in the former. Therefore, one cannot speak of hyperleukocytosis which takes place exclusively in the venous area.

It is assumed to be brought about by a direct formation of white blood cells in the blood. Together with Löwit, we must deny this absolutely. Our microscopic examinations of the processes of regeneration of leukocytes during hyperleukocytosis, as well as the behavior of blood-producing organs, cannot be considered as concluded, yet we cannot gain any clues from them nor from those conducted by Römer that generation takes place in the flowing blood. Our findings agree with Löwit that, after hypoleukocytosis mononuclear cells are found exclusively and only after hyperleukocytosis sets in, do the polynuclear ones gradually outnumber the others. With regard to the latter fact, Löwit did not attach enough importance to one fact, namely, that the polynuclear leukocytes found during hyperleukocytosis are eosinophilic. Therefore, we believe that these polynuclear cells did not all come from the conversion of newly introduced mononuclear ones into polynuclear cells, but that in part old leukocytes lying ready for disposal in the bone marrow enter the circulating

blood as a consequence of the chemical stimulus of the injection. We will speak about this chemical stimulus in more detail later on. Here we would only like to explain one more finding; the formation of thrombi with leukocytes, on which Römer founds his theory, often found in the vessels at various changes of leukocytes. This adhesion of white blood corpuscles in masses, occasionally observed by us, as well as by Everard Demoor, and Massart, can best be interpreted with our findings by the fact that they are singular thrombi, washed out of the capillaries like those which were found in the lung preparations. Römer's hypothesis after all is rejected as /437 an explanation of the formation of additional leukocytes found in hyperleukocytosis.

Finally, we come to Löwit's theory which he so elaborately worked out and seems so enticing being based on so many experimental results. Reinforced by the verification of more recent works, like ours, it appears that hypoleukocytosis precedes hyperleukocytosis, seemingly substantiating the immediate connection between the two phenomena which Löwit values so highly. According to Löwit, this connection exists to the extent that the latter phenomenon could not exist at all without the former.

In a series of experiments, we have shown that under special experimental modifications primary hyperleukocytosis can be produced. We will not go into these experiments now, but shall examine the reasons why Löwit objects to the chemotactic viewpoint. Löwit denies that chemotaxis has any influences on the process of leukocytosis. On the other hand, he expressly recognizes that he cannot explain why leukolysis gives rise to such a high-grade release of leukocytes into the blood, an overflow of immature cells from the blood-forming organs. For what reasons does Löwit deny the chemotactic influence on these phenomena? The first and main reason is that in his conclusions he pays attention to the decay of the leukocytes, which is supposed to be identical with the appearance of hypoleukocytosis by injection. We hope we have discussed in sufficient detail the reasons why this assumption is erroneous. The main argument of Löwit's theory is thereby eliminated: Hyperleukocytosis is not caused by decay. Furthermore, Löwit argues against chemotactic

influences, stating that it is possible to produce hyperleukocytosis by cooling, shock, etc., which do not contain chemically effective substances which, when introduced into the blood, can act upon the blood-forming organs. The objection which Löwit raises against his own reasoning — that hypoleukocytosis preceding hyperleukocytosis and accompanying the decay of white blood corpuscles, Horbaczewski's nucleic leukocytosis, might give rise to hyperleukocytosis — is also rejected by us. And for the same reason as does Löwit: There is no deterioration after cooling and shock, but the leukocytes are held back in certain places of the vascular system, causing hypoleukocytosis. Therewith, the evidence against chemotaxis used by Löwit in this /438
respect is eliminated. Finally, we would like to point out the experiments once again where we were successful in achieving hyperleukocytosis without preceding hypoleukocytosis, that is, hyperleukocytosis, as demanded by Löwit, "which is initiated by chemical attraction of white blood corpuscles into the blood as a consequence of certain substances in the blood serving as a lure."

In discussing the various hypotheses on leukocytosis and rejecting the reasons given by the particular authors, we have already mentioned those which support the conditions that allow hypo- and hyperleukocytosis. Now we must add some data from our experimental material and from the literature in order to support our assumptions.

Among the papers on chemotaxis which touch upon our area, the work by Pfeffer [26] has to be mentioned above all. His concept of positive and negative chemotropism, especially in regard to the process we are dealing with here, is of greatest importance. The peculiar effect certain substances have on plant and animal cells, elucidated by the significant work by Pfeffer and Stahl, was shown for leukocytes also. From various sides, Leber, [27] Massart and Bordet [28], Pekelharing [29], Hüppe and Scholl [30], v. Limbeck, Rieder, Steinhaus [31], Gabitschewsky [32], Stahl and Stange, Ribbert, Hess [33], and Lubarsch, Everard, Demoor and Massart, Bouchard [34], Metchnikoff, Michelson, Botkin [35], Emelianov, Hertwig [36] and others, the importance of certain substances introduced into the organism for the leukocytes was pointed out. It was shown that it was not the microorganisms or — as Werigo,

for instance, maintained for part of the phenomena — only corpuscular elements which caused the appearance of chemotaxis, but that the metabolic products of bacteria can do this to a much greater degree. This property was then claimed for other substances, too. We believed the organ extracts of spleen, bone marrow and thymus gland had an effect of leukocytosis. The actual phenomena produced by the aforementioned extracts have already been discussed in detail, and especially our assumption of two processes involved in these activities has been proven. In the attempt to clarify the influences of these substances on the white blood corpuscles, we wish to interpret them along with Hertwig, such as to assume a certain threshold value. Thereby, the various phenomena which occur after the particular injections can be understood most easily when in such great variety. Consequently, we would have to distinguish the following conditions:

1. When a substance is introduced into the organism in small doses, especially into the blood stream, it will exert a small rejecting effect on the leukocytes because of its small concentration. Repeating these small doses in certain time intervals brings out the attractive force more and more; every single injection has a stimulating effect on the blood-producing organs, and the final effect is one of considerable hyperleukocytosis (Experiment 89, 90).

2. When we inject a moderately strong dose (for our extracts 6 to 8 cc) a capillary attractive effect on the leukocytes is produced; they are crowded into the capillaries and held there. Gradually, the situation is reversed; the particular substance has diffused into the liquid mass with its high concentration gradient; it has lost its rejecting power so that now it can stimulate the blood-producing organs to release the new elements and expell the stored elements.

3. For a third case, an excessively high dose is administered. Naturally the rejecting force must be very high at first. On the other hand, we have introduced into the blood stream such a large quantity of substance that it should have an effect on the blood-producing organs within a short

time. First, of course, the newly produced cells are rejected and forced into the capillaries, but soon the effect on the organs forming the blood cells is so great that hyperleukocytosis sets in a relatively short time.

We still have to discuss a series of side questions which result from the experiments of the second to last chapter, especially the changes in the appearances when, during the various stages of the phenomena caused by a single injection, another injection is made. First, we saw that hypoleukocytosis is considerably prolonged when a second injection of equal strength is made at that time. The rejective effect of the first dose on the leukocytes still continues, and a new dose adds new momentum to the rejection. It is reinforced and prolonged, delaying the process which attracts the leukocytes more than usual.

This consideration leads directly to the interpretation for the second /440
modification of our experiments: when another injection is given during increasing hyperleukocytosis — namely, at a time when the positive chemotactic effect is in full force — the new injection halts it only slightly, and after a short time it increases it considerably.

It is also easily understood that after a new injection given after the state of hyperleukocytosis has been present for some time and the positive chemotactic force has been spent already, hypoleukocytosis will once again occur. Nevertheless, the trend toward hyperleukocytosis continues; the new injection can influence and interrupt it only to a moderate degree. Already after a relatively short time span, the number of leukocytes will once again increase.

The result of experiments 83, 86, in which we observed a high grade but brief hypo-, and later on, a very considerable hyperleukocytosis, after injecting an excessively large dose (20 cc at once) can probably only be interpreted to mean that here the positive chemotactic effect sets in much more rapidly than under normal conditions. First, because of the high dose, the effect which holds the leukocytes in the capillaries must be very

pronounced. As soon as this has happened and the injected substance has diffused into the pulp, and entered into the lymphatic system, an even stronger attraction of the white blood corpuscles away from the organs which produce them will arise.

Experiments 87, 88 also speak very much in favor of the chemotactic concept. First a small dose was injected, so that it would be present in the pulp in low concentrations when the second usual dose of 8 cc was given, because, based on the known chemotactic principles, the effect of the second injection should be considerably influenced. The result showed the correctness of our assumption: after the second injection, there was only a slight reduction in the number of leukocytes which was followed by a continual strong increase.

Most remarkable were the last test results; they clearly oppose Löwit's concept of the nature of leukocytes; on the other hand, they represent an important argument for concept. Experiments 89, 90 showed that with certain modifications of the experiment, it is possible indeed to avoid completely hypoleukocytosis which, after injections, usually precedes hyperleukocytosis and to produce the latter primarily. With these results Löwit's /441 law stipulating there is a definite connection between hypo- and hyperleukocytosis, the latter being impossible without the former, has been severly shaken. According to the principles developed above, it is clear that the small amounts of injected substance each by itself is not able to exert an energetic effect upon the leukocytes, and is already distributed in the pulp before it could cause strong retention of leukocytes in the capillaries. After we repeatedly inject these small doses in certain time intervals, the pulp after a short time contains such a quantity of a certain substance that the positive chemotactic process far outweighs the negative one which, due to the small doses, remained small. The balance maintained for some time is now upset in favor of the first process, and we claim that this is evidence of hyperleukocytosis.

We stress several other points from our test results, for instance, experiment 36-38 with nucleic acid. Nothing can substantiate our view better than these results. Löwit already in his book points out that he did not use nucleic acid as a substance for injection, because it had proven to be too poisonous. Through the kindness of Professor Kossel, we received a pure preparation and found the following:

To a dose of 0.2 the experimental animal reacted similarly as to normal substances for injection; to a double dose — with hyperleukocytosis which set in only after 28 hours, and to 2-1/2 times the dose — with a reduction 26-1/2 hours after the injection, but a low-grade hyperleukocytosis only after 70 hours. This clearly points to two processes involved in the appearance of leukocytosis, a negative one and a positive one. After a small dose of nucleic acid, the latter predominates; after 2-1/2 times the dose, the former predominates for days.

This brings us to the processes which take place when leukocytosis changes are observed in clinical cases and about which we would like to say a few words. We shall completely ignore the question of immunity in which the leukocytes, according to some researchers, play such an important part due to their phagocytic properties. Relative to phagocytosis, we would like to point out one thing, which can be decided upon now. Bacteria and metabolic /442 products are not the only substances which produce hypo- and hyperleukocytosis, but, as we saw, a whole series of other substances. What relationship the leukocytes have to the bacteria is still an unsolved puzzle. Four possibilities are conceivable:

1. According to Metchnikoff and his pupils, they only play the part of phagocytes. As soon as an animal organism is attacked by bacteria, they fall on them as destructive agents. If they are victorious in this battle, if they dominate the bacteria, then the organism recovers. If not, the bacteria gain the upper hand, and the invaded body succumbs.

2. The leukocytes could only have the function of "transporters". This is Wyssokowitsch's opinion. He thinks that the white blood corpuscles are only designed to bring the bacteria to the endothelial cells, but the act of devouring is exclusively their function.

3. Hess assumes a third possibility; the leukocytes possess both abilities: transporting and devouring.

4. In contrast to the above three views, there is a fourth one which has many followers — namely, that the leukocytes are not significant at all in the fight with bacteria, and that the symptoms observed in infectious diseases of leukocytosis changes are just incidental.

For the time being, we confine ourselves to characterizing the four possibilities at this time, which could be possible in regard to the behavior or leukocytes toward bacteria. We shall now go on to the clinical cases. Here also we have to start with a qualification which we cannot emphasize enough. As far as the change of leukocytosis in man is concerned, our conclusions cannot be cautious enough. In the previous chapters, we have shown how many different factors can change the number of leukocytes, and since we naturally cannot take blood from man, especially when he is sick, as often as from an experimental animal, and also since the process of the sickness extends over days and weeks, considerable differences in leukocytosis can easily escape detection. Nevertheless, we are now entitled to make a safe judgement on the general leukocytosis condition in most diseases, because of the large number of blood counts which have been performed in the /443
various clinics during the course of recent years. Today we know that most infectious diseases, especially febrile ones, go hand in hand with more or less high hyperleukocytosis, except abdominal typhus, malaria and in the majority of cases the nonlocalized puerpural sepsis (pure septicemia). In the diseases last mentioned, there is frequently hypoleukocytosis, in contrast to the first mentioned. But this is the case also in the remaining infectious diseases when they are terminal. In particularly serious cases which terminate after a few days, there is no increase detectable during their course;

however, there is a steady decrease of white blood corpuscles. The question is now: can these clinical findings of changes in leukocytosis be made to agree with our artificial ones, is our concept of the presence of two processes and further hypotheses about chemotactic influences on the white blood corpuscles justifiable? We think we can answer these questions affirmatively.

First, let us look at hyperleukocytosis in injectious diseases, Rieder's "inflammable leukocytosis". He and v. Limbeck attribute to it the same chemotactic influences, and share our opinion. Löwit, however, disputes it, and believes also that the hyperleukocytosis found here is preceded by leukolysis, only it has not been detected. We admit that indeed there may be hypoleukocytosis in the beginning of the illness in single cases, and would be detected if a count were made at the right time. The primary reduction of white blood corpuscles, however, is not a requirement for hypoleukocytosis. On the contrary, we believe to have found a clue from our experimental results for the origin of leukocytosis in most illnesses. Even if bacterial invasion is more or less acute, it probably still is not a sudden increase in large quantities. Rather the nature of the infection is probably the multiplication of germs in the body, and over a long period of time small quantities of the particular bacteria continually get into the liquid mass, similar to our experiments 90, 91, where we also by repeated introduction of small doses were able to produce a directly increasing hyperleukocytosis. When the bacteria and their metabolic products are destroyed, and we disregard the force which accomplishes this, then there is no more reason for further attraction of blood cells from the organs which produce them.

But the situation is completely different for leukocytosis when the /444
infection is severe from the start, or becomes abrupt and leads to death. In both cases one finds a distince reduction of white blood cells as is known from counts made in the hospitals, but for hypoleukocytosis — almost never. This, however, is not true when pus formation is involved.

Thus, we saw in several experiments that an animal which had received strongly concentrated bacterial poison or was intoxicated with nucleic acid would die in a state of hypoleukocytosis. This phenomenon can be explained by the chemotactic theory. In sufficient dilution the material attracts the white blood corpuscles, but repels them after a certain concentration, or also it may paralyze the movement and proliferation.

There is much to be said for the latter assumption.

While one usually finds hypoleukocytosis in pure septecemic Sepsis puerperalis, the white blood corpuscles are almost always increased as soon as purulent localization sets in, e.g., peritonitis or para metritis. Probably the absence of hyperleukocytosis and suppuration is due to a common reason which is the damaging influence on proliferation and locomotion of the leukocytes.

We do not want ot extend this discussion to all the other forms of hyperleukocytosis which Rieder mentions in his book: such as cachectic, praeogonal, hemorrhagic and physiological; digestive, infant, pregnancy hyperleukocytosis. For all these forms, one could assume, as we mentioned for the inflammable case, the presence of either both processes, or of one alone. On the other hand, we do not contend that there could not also be other factors which could exert special influences on the change of leukocytosis; this is especially true for praeogonal hyperleukocytosis which is difficult to explain. These questions will only be answered with certainty when we know more about the nature of chemotaxis and when, furthermore, the biological puzzles which at this time still exist concerning the origin, life and death of the white blood cells are solved. There is only one more question we would like to briefly touch upon, because our findings contradict those of another author and show once more how carefully one has to consider changes in leukocytosis, because too few counts can easily lead to incorrect conclusions. Winternitz [37] states that hyperleukocytosis can be produced by cool baths. This statement requires considerable qualifications: first, the immediate effect is hypoleukocytosis; only after that does

hyperleukocytosis occur. First of all, we tested these processes on experimental animals and reported the results in Chapter II. Furthermore, we were able to make observations on a clinical case. Immediately after a cool bath, the hyperleukocytosis which existed otherwise dropped considerably, and only after several hours did it rise again to its full extent and above.

Winternitz' experiments which, by the way, Grawitz [38] has already criticized, cause us to remark in general that hyperleukocytosis may in some cases only seem so, but is actually caused by a thickening of the blood. To anticipate the objections to this, we counted in many experiments the erythrocytes, and using their normal value, established that in our cases we were not dealing with thickened blood.

Finally, we would like to give a few words on the injection of organ extracts which we have used in large quantities for various illnesses during the last year at the Clinic for Internal Medicine. The value we attribute to this treatment will not be discussed further at this point. Recently we have expressed our views in the "Verein für innere Medizin", and, therefore, can refer to the particular proceedings.

It should only be mentioned here, relative to the area of leukocytosis discussed here, that we were able to produce the same effects of hypo- and hyperleukocytosis in man as in experimental animals by subcutaneous injection of organ extracts from spleen, bone marrow, thymus gland. On the other hand, the preparations that were found ineffective in animals like pancreas, kidney, thyroid, liver extracts also had no influence in man on leukocytosis. Moreover, it is of special interest to note that febrile patients who received the injections usually reacted with temperature variations. Thus, the patients with lung tuberculosis reacted quite similar to treatment with tuberculin. Local reactions in the case of lupus and tuberculosis of the throat were not observed. Similarly, we did not succeed in producing hyperleukocytosis by injection in infectious diseases, typhus and pure septicemic sepsis puerperalis — up till now we did not have the opportunity to observe a case of malaria.

For the time being, we shall not answer the question as to what part is played by changes in leukocytosis produced artificially by injection. Our experiments are not sufficient to reach a definite conclusion at this time; with the selection of clinical cases for the injections, we were very cautious. Even though the injections which were made under aseptic precautions and with material that was really sterile in general had no damaging influence, it would nevertheless be conceivable to produce hypoleukocytosis by injection, i.e., an enormous accumulation of leukocytes in the lung capillaries. Furthermore, the temporarily altered clotting ability of the blood is to be taken into account. /446

With the extract of organs so frequently used now for treatments, these circumstances should not be disregarded.

REFERENCES

1. Reinert, E. Die Zählung der Blutkörperchen und deren Bedeutung für Diagnose unde Therapie (Counting of Blood Corpuscles and Their Significance for Diagnosis and Therapy). Von der medicin. Facultät zu Tübingen gekrönte Preisschrift. Leipzig, 1891.

2. Rieder. Beiträge zur Kenntniss der Leukocytose und verwandter Zustände des Blutes (Contributions to the Knowledge of Leukocytosis and Related Conditions of the Blood), 1892.

3. Jacob. Ueber arteficielle Hyperleukocytose (On Artificial Hyperleukocytosis). Verhandlungen der Physiologischen Gesellschaft zu Berlin. 1893.

4. Goldscheider. Weitere Mittheilungen über die Leukocytenfrage (Further Communications on the Question of Leukocytes). Verhandl, der Physiologischen Gesellschaft zu Berlin. 1893.

5. v. Limbeck. Klinisches und Experimentelles uber die entzündliche Leukocytose (Clinical and Experimental Things on Inflammatory Leukocytosis). Zeitschrift für Heilkunde, Vol. X.

6. Römer. Ueber den formativen Reiz der Proteine Buchner's auf Leukocyten (On the Formative Stimulus of Buchner's Proteins on Leukocytosis). Berliner klin. Wochenschr. 1891.

7. Römer. Die chemische Reizbarkeit thierischer Zellen (The Chemical Excitability of Animal Cells). Virchow's Arch. Vol. 128.

8. Buchner. Die chemische Reizbarkeit der Leukocyten und deren Beziehung zur Entzündung und Eiterung (The Chemical Excitability of Leukocytes and Their Relationship to Inflammation and Supuration). Berliner klin. Wochenschr. 1890

9. Buchner. Ueber pyrogene Stoffe in der Bakterienzelle (On Pyrogenic Matter in the Bacterial Cell). Berliner klin. Wochenschrift, 1890.

10. Löwit. Studien zur Physiologie und Pathologie des Blutes und der Lymphe (Studies in Physiology and Pathology of the Blood and the Lymph). Jena, 1892.

11. Schulz. Experimental Examinations of the Occurrence and Diagnostic Significance of Leukocytosis. Arch. f. klin. Med. Vol. 51, No. 2/3, 1893. /447

12. Römer. Darstellung und Wirkung proteinhaltiger Bakterienextracte (Preparation and Effect of Proteinaceous Bacterial Extracts). Berliner klin. Wochenschrift, 1891.

13. Massart, Everard, Demoor. Sur les modifications des leucocytes dans l'infection et dans l'immunisation (On the Modifications of Leukocytes in Infection and Immunization). Annales de l'Institut Pasteur. Vol. VII.

14. Wérigo. Les globules blancs comme protecteurs du sang (The White Blood Cells as Protectors of the Blood). Annales de l'Institute Pasteur. 1892.

15. Müller, R.F. Ueber das Verhalten der Leukocytose nach Bakterieninjectionen (On the Behavior of Leukocytosis After Injection of Bacteria). Inaug. Dissertation. Berlin, 1894.

16. Michelson. Experimentelle Studien über Lymphagoga (Experimental Studies on Lymphagoga). Inaugural-Dissertation. Dorpat, 1892.

17. v. Samson-Himmelstjerna. Experimentelle Untersuchungen über das Blut in physiologischer und pathologischer Beziehung (Experimental Investigation of the Blood in Physiological and Pathological Respects). Inaugural-Dissertation. Dorpat, 1882.

18. Groth. Ueber die Schicksale der farblosen Elemente im kreisenden Blute (On the Fate of the Colorless Elements in Circulating Blood). Inaugural-Dissertation. Dorpat, 1884.

19. Horbaczewski. Untersuchungen über die Entstehung der Harnsäure im Säugethierorganismus (Investigations on the Origin of Uric Acid in the Organism of the Mammal). Sitzungs-Berichte der Akademie der Wissenschaften zu Wien, Vol. 98, No. 3.

20. Horbaczewski. Beiträge zur Kenntniss der Bildung der Harnsäure und der Xanthinbasen, sowie der Entstehung der Leukocytosen im Säugethierorganismus (Contributions to the Knowledge of Formation of Uric Acid and the Xanthin Bases, as Well as the Origin of Leukocytosis in the Organism of Mammals). Sitz.-Ber. der Akad. d. Wissensch. zu Wien, Vol. 100, No. 3.

21. Jacob and Krüger. Ueber Harnsäure-, Xanthinbasenausscheidung und Leukocytose bei einem mit Organextracten behendelten Falle von Leukämie (On Uric Acid and Xanthin Base Excretion and Leukocytosis in a Case of Leukemia Treated with Organ Extracts). Deutsche med. Wochenschrift, 1894.

22. Emilianow. Sur la rôle de la rate au point de vue de la composition morphologique du sang et sur l'influence de l'exstirpation de cet organe sur la moelle des os (On the Significance of the Spleen from the Point of View of the Morphological Composition of the Blood and on the Influence of Extirpation of This Organ on the Bone Marrow). Arch. des sciences biol. Petersbourg. T. 2. 1893.

23. Zesas. On the Extirpation of the Spleen in Man and Animal. Langenbeck's Archiv f. Chir. Vol. 28, 1883.

24. Credé. Exstirpation der kranken Milz am Menschen (Extirpation of the Diseased Spleen in Man). Gesellsch. f. Chirurgie. XI. Congress, 1882.

25. Lassar, O. On the Cold. Virchow's Archiv., Vol. 79, 1880, p. 168.

26. Pfeffer. Untersuchungen aus dem botan. Institut in Tübingen (Investigations from the Bot. Inst. in Tübingen).

27. Leber, Th. On the Origin of Inflammation and the Effect of the Harm Causing the Inflammation. Fortschr. d. Med., 1888, p. 460.

28. Massart et Bordet. Annales de l'Institut Pasteur. 1891.

29. Pekelharing. Semaine méd., 1889.

30. Hüppe and Scholl. Berliner klin. Wochenschrift, 1891.

31. Steinhaus. Die Aetiologie der acuten Eiterungen (The Etiology of Acute Suppuration). Leipzig, 1889.

32. Gabritschewsky. On the Chemotactic Properties of Leukocytes. Annales de l'Institut Pasteur, 1890.

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33. Hess. Untersuchungen zur Phagocytenlehre (Investigations in Phagocytology). Virchow's Archiv., Vol. 109.

34. Bouchard. Internationaler Medicine Congress. General Session, 1890.

35. Botkin. Hämatologische Untersuchungen bei Tuberculose (Hematological Investigations in Tuberculosis). Deutsche medicin. Wochenschrift, 1892.

36. Hertwig. Ueber die physiolog. Grundlage der Tuberculinwirkung (On the Physiological Basis of the Effect of Tuberculin). Jena, 1891.

37. Winternitz. New Investigations of Blood Changes After Thermal Treatment. Centralbl. f. klin. Medicin., No. 49, 1893.

38. Grawitz, E. Remarks to the Article etc. by Dr. W. Winternitz. Ebendort, No. 2, 1894.